AN INVESTIGATION OF THE PATHOGENESIS AND PATHOMORPHOLOGY OF BLACKJACK OAK (*QUERCUS MARILANDICA*) LEAF TOXICITY

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LIST OF SYMBOLS

abs	absolute
alb	albumin
ATP	adenosine triphosphate
Band	band neutrophil
Baso	basophil
Bili. T.	total bilirubin
BSA pptd/ mg leaf	bovine serum albumin precipitated per milligram of leaf
BSA	bovine serum albumin
BUN	blood urea nitrogen
BW	body weight
Ca	calcium
Cl	chloride
cm	centimeter
Creat	creatinine
DW	dry weight
EDTA	ethylenediamine tetra-acetic acid
Eosin	eosinophil
FE_{Na}	fractional sodium excretion
Fig	figure
g	gram
g/dl	grams per deciliter
GFR	glomerular filtration rate

g/kg/day	grams per kilogram per day
Hct	hematocrit
H&E	hematoxylin and eosin
Hgb	hemoglobin
IU/L	international units per liter
I.V.	intravenous
K	potassium
kg	kilogram
L	liter
Lym	lymphocyte
mEq/L	milliequivalents per liter
mg/dl	milligrams per deciliter
mg/kg	milligrams per kilogram
mg/ml	milligrams per milliliter
ml/kg	milliliters per kilogram
ml/kg/day	milliliters per kilogram per day
ml/min	milliliters per minute
mmol/kg	millimoles per kilogram
um	micrometer
Mono	monocyte
Na	sodium
nm	nanometer
Osm	osmolality
PAH	para-aminohippuric acid
P_{Cr}	plasma creatinine
%	percent
phos	phorphorus

P_{Osm}	plasma osmolality
RER	rough endoplasmic reticulum
Seg	segmented neutrophil
SG	specific gravity
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
T.P.	total protein
TRBF	total renal blood flow
UA/LC	uranyl acetate/lead citrate
U_{Cr}	urine creatinine
units/kg	units per kilogram
U_{Osm}	urine osmolality
v/v	volume to volume
WBC	white blood cell count
$\bar{x}$	mean

CHAPTER I

Introduction

Poisoning of domestic livestock resulting from ingestion of oak (Quercus spp.) buds, twigs, leaves, and acorns is generally an infrequent, yet serious, condition encountered in veterinary medicine. More than 60 different species of oak have been identified in North America, all of which should be considered potentially toxic to livestock (1). Oak poisoning has been recognized since at least 1662 where it was stated, "Again oak leaves, if sheep eat thereof green, it is evil for them, especially young lambs, which will kill them; and likewise of other cattel." (2, p. 243). No further mention of oak poisoning can be found in the literature until 1893 (3). More recently, oak poisoning has been reported as a regionally important, but sometimes widespread, condition of livestock, affecting cattle, horses, and sheep.

Due to widespread occurrence of oak poisoning, further study of this condition is warranted. The purposes of this investigation were:

1. To develop a model of oak poisoning in an animal species that is less expensive and which requires a smaller dose of leaves than do cattle.

2. To compare renal clearance values and clinical pathology data of oak poisoned animals with that of existing animal models of acute renal failure in an attempt to elucidate the pathogenesis of the renal lesion resulting from oak leaf poisoning.

3. To document the chronologic progression of the renal lesion by light and electron microscopy.

CHAPTER II

Review of the literature

Introduction

Oak poisoning of livestock has been reported in the United States (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18) and Europe (19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32). Natural cases of poisoning have been reported in cattle (4, 5, 6, 8, 9, 10, 11, 13, 14, 16, 17, 18, 22, 24, 25, 26, 27, 28, 29, 30), sheep (5), and horses (7, 20, 21, 23, 31, 33). Experimental poisoning by oak or its components has been reported in rabbits (34, 35, 36, 37, 38, 39, 40), goats (41, 42, 43, 44), rats (45, 46, 47, 48), mice (45), cattle (37, 49), sheep (9, 37), and guinea pigs (37). Clinical signs, clinical pathology, and gross and histopathologic lesions are similar in most species.

Oak Species Reported as Toxic

Only a small number of oak species have been reported as toxic. Quercus havardi has been reported as toxic in cattle (37, 50, 51, 52) and in rabbits (36, 37, 38, 40). Quercus gambellii has been reported to be toxic in cattle (5). Cattle and sheep were reported to be subject to poisoning by Quercus breviloba (5). European literature has most commonly mentioned Quercus robur as the cause of oak poisoning in cattle (24, 29). Cattle and sheep have been poisoned by Quercus lobata (9). Cattle have died from ingestion of Quercus incana (51). Other species

which have been considered toxic are Quercus stellata (37), Q. velutina (1), Q. prinus (1), Q. rubra var. borealis (1), and Q. coccinea (1). Most reports have not identified the species of oak suspected in the intoxication.

Common Circumstances in Oak Poisoning

In many outbreaks of oak poisoning, several common circumstances are shared. All conditions are not present in all cases, but many are frequently noted during outbreaks:

1. Outbreaks often occur when little forage is available to livestock. Poisoning by oak leaves and buds tends to occur in the spring (5, 11, 27, 52), whereas poisoning by acorns tends to occur in the fall (9, 10, 18, 21, 22, 24, 27).

2. In years that poisoning occurs due to ingestion of acorns, the acorn crop is often unusually heavy (9, 10, 21, 22, 24, 27).

3. Soil conditions may affect the toxicity of the oak plant (11).

4. Mature acorns are usually reported as the toxic agent except where severe weather provides green acorns at ground level (10).

5. Green acorns may be less palatable (45) but are more toxic (9) than ripe acorns.

6. Buds and young leaves are more toxic than mature leaves (40), but toxicity due to older leaves has been reported (53).

7. More poisonings occur in animals younger than 2 years (6, 10, 13, 14) than occur in animals older than 2 years old) (9, 16).

8. Certain animals acquire an appetite for acorns and will eat them preferentially to other food sources (4, 25).

Components of Oak Leaves, Buds, and Acorns

Crude extracts of oak buds, leaves, and acorns yield a group of chemically similar substances collectively named "tannins". Several distinct chemicals have been isolated from these tannins, and the compounds have been identified and tested for toxicity in animals.

A crude tannin extract was produced by extracting mechanically ground oak buds and leaves with 80° C water, followed by alcoholic washing and drying (40). Chemical analysis of the extract identified 1 component as gallic acid; thus, the extract was designated a gallotannin. In 2 separate experiments, rabbits given this gallotannin via stomach tube developed clinical signs and lesions consistent with those observed in cases of natural oak poisoning in other species (36, 40).

Constituents of acorns have been individually tested in laboratory animals (45). It was shown that ellagic acid, gallic acid (3,4,5-trihydroxybenzoic acid), quercitol (cyclohexanepentol), quercitrin, and an unidentified alkaloid were nontoxic when given intraperitoneally to mice. In the same experiment, tannic acid was toxic to mice when administered intraperitoneally.

Nearly identical gross lesions were produced in rabbits when they were administered oral gallic acid, pyrogallol, tannic acid, or fresh buds and leaves of Quercus havardi. These lesions were consistent with those of oak poisoning in other species (38).

Tannic acid and its constituents, gallic acid and pyrogallic acid, have a wide variety of effects in herbivorous animals (54, 55). These effects include inducing:

1. Vitamin B12 responsive macrocytic anemia.
2. Lymphocytosis in peripheral blood.

3. Inhibition of the number and maturation of myeloblasts, promyelocytes, and myelocytes producing a granulocytopenia.

4. Inhibition of thrombocytopoiesis, producing a thrombocytopenia.

5. Reduction in ruminal bacterial flora with concurrent decrease in vitamin B12 production.

6. Reduction in responsiveness to acetylcholine and histamine by the stomachs (glandular and nonglandular) and small intestine (tannic acid only).

7. Inhibition of rumen urease activity.

Clinicopathologic Presentations of Oak Poisoning in Various Species

Cattle--Clinical signs and lesions, although somewhat variable from case to case, are generally similar (4, 6, 10, 13, 14, 16, 17, 22, 24, 25, 29, 49, 56). Typically, affected cattle are depressed, anorectic, afebrile, and have a serous to mucoid nasal discharge. Rumen motility is decreased or absent and the rumen contents foul smelling and stratified. Heart and respiration rates are variable. Animals are usually constipated at the onset of signs and the hard fecal balls are covered by large amounts of mucus. Constipation may later change to mucoid or bloody, foul-smelling diarrhea. Many affected cattle have polydypsia and polyuria, producing large quantities of dilute urine.

Cattle commonly die within a few days to a month but occasionally die suddenly. Animals which survive the acute condition may return to normal. Chronically affected animals become progressively more dehydrated and emaciated and may have signs for several months.

Blood urea nitrogen values are generally increased in affected

animals (often over 150 mg/dl), and blood calcium values are low as are the packed cell volumes. Urinalysis usually reveals low urine specific gravity (as low as 1.005), acid pH, and positive tests for urine glucose and protein (between 30 and 100 mg/dl). The SGOT of affected animals may be elevated.

Only the sodium sulfanilate clearance test has been reportedly used to monitor oak-poisoned animals (9). The reported renal clearance (half-life) of this drug in cattle was 22 minutes. The renal clearance (half-life) in an oak-poisoned cow was 2 1/2 hours. The accuracy or usefulness of this test cannot be determined because only 1 oak-poisoned animal was tested.

The ratio of total to free cholesterol has been used to monitor liver function in animals poisoned by oak (56). In animals which have developed clinical signs of oak poisoning, the level of free cholesterol rises; the esterified cholesterol level drops; but the total cholesterol level remains normal. This indicates a decrease in liver function (decrease in ability to esterify cholesterol) which is due to the toxic action of oak.

Gross lesions in acute cases include foul-smelling rumen content, catarrhal-hemorrhagic abomasitis and enteritis, hydrothorax, hydropericardium, hydroperitoneum, and perirenal and mesenteric edema (4, 10, 53). Kidneys are usually edematous, pale, and have petechial hemorrhages scattered beneath the capsule. Animals surviving the acute stage have varying degrees of emaciation and subcutaneous edema. The kidneys are often small, pale grey-brown, and have a pitted or mottled surface. The abomasum or serosal surfaces may have an ammoniacal odor, and oral and esophageal ulceration and rumenitis may be evident.

The most commonly described histologic lesion is groups of proximal convoluted tubules undergoing tubular epithelial degeneration and necrosis (6, 9, 22, 52, 53). Mildly affected tubules have swollen epithelial cells with rarefied, granular cytoplasm and occasional fat vacuoles. More severely affected tubules are lined by dead epithelial cells which have pyknotic or karyorrhectic nuclei and deeply eosinophilic cytoplasm. Many tubules are dilated and contain pink, smooth to granular protein or cellular casts. Tubules are often separated by interstitial edema. Chronic renal lesions include tubular regeneration, dilated tubules lined by flattened epithelia, diffuse and multifocal lymphocytic accumulations in the cortex and medulla, and periglomerular and diffuse interstitial fibrosis. Hepatic lesions have not been described in cattle.

Sheep--Poisoning of sheep by oak has been documented and closely resembles the condition in cattle (5).

Clinical signs include listlessness, anorexia, decreased rumination, and a sticky nasal discharge. Pulse and respiration rates are increased; the animals are normothermic and often have dependent subcutaneous edema. Early in the disease, sheep are constipated, having mucus and blood-covered fecal balls. Affected sheep often have polyuria and hyposthenuria. Chronic cases often develop a fetid diarrhea. Sheep are more likely to die acutely than are cattle but may be ill for up to 1 month before death occurs.

Gross lesions in sheep resemble those in cattle and include ascites, hydropericardium, abomasal and small intestinal hemorrhage and edema, and an erosive colitis. The kidneys are pale and have petechial hemorrhages scattered throughout.

The microscopic renal lesion of oak poisoning in sheep is similar to that of cattle (9).

Horses--Horses have been poisoned by mature green oak leaves (7) and by acorns (20, 21, 23, 31, 33). Similar signs and lesions are produced by either source of oak.

Affected horses often are depressed, anorectic, have reduced intestinal peristalsis and abdominal pain. Mucous membranes may have injected vessels and may be icteric. Mildly affected horses may be constipated, whereas severely affected horses may have fetid diarrhea. Impaction may occur if large numbers of acorns are eaten.

The most striking gross lesion in the horse is severe edema of the large intestine and colon, the wall of which may be 3 centimeters thick. The mucosa of both the large and small intestines may be hemorrhagic and ulcerated. A liver lesion has been described as "advanced liver damage" in a case of chronic oak poisoning (20). Kidneys are characterized grossly as being pale, swollen, and having corticomedullary streaking. Histologically, the kidneys have moderately severe, diffuse tubular degeneration and necrosis (33).

Rabbits--Rabbits with orally administered gallic acid, pyrogallol, tannic acid, leaves of Quercus havardi (38), or crude tannin isolates (36, 40) developed signs and gross lesions similar to those of oak-poisoned cattle. Consequently, the rabbit has been proposed as a model of oak poisoning in cattle and sheep.

Rabbits given oak leaves or their components became anorectic and lethargic. Diarrhea was often seen before death. Affected rabbits had an increased packed cell volume, hemoglobin, serum glutamic oxaloacetic

transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and blood urea nitrogen (BUN). Serum albumins and globulins, total lipids, and cholesterol were unchanged. Beta glucuronidase activity of the liver was increased which is interpreted as a normal detoxification mechanism.

Grossly, the liver was mottled, and the kidneys were swollen, pale, and occasionally hemorrhagic or congested. The stomach was hemorrhagic and frequently ulcerated. The intestines were commonly hemorrhagic.

The liver had severe necrosis and loss of glycogen histologically. Renal tubular epithelial cells were vacuolated, and the tubules occasionally contained dead epithelial cells (36).

Ultrastructural Changes Attributable to Tannic Acid

Ultrastructural changes attributable to parenteral administration of tannic acid have been documented in rats and rabbits. Parenteral administration of tannic acid in rats has caused zonation of hepatocytic nucleolar ribonucleoprotein, the significance of which is unknown (47). Rats given tannic acid parenterally also had disaggregation of hepatocytic polyribosomes with concurrent reduction of incorporation of amino acids into cellular protein (48).

Rabbits given tannic acid parenterally developed both renal and hepatic lesions. Renal lesions included increased cytoplasmic vesicles, swelling of the cytoplasm, accumulation of lipid droplets, and death of proximal convoluted tubular epithelia (35). Hepatic lesions included disorganization of rough endoplasmic reticulum, polyribosomal disaggregation, accumulation of lipid droplets, and cellular death (34).

The overall applicability and significance of these results were unknown. Changes in the rabbit were considered nonspecific lesions of cell intoxication and death, not specific lesions of tannic acid poisoning (34).

Normal Protective Mechanisms Against Oak Poisoning, Prevention and Treatment

Oak poisoning in cattle can be prevented by natural mechanisms in many cases. Oak leaves eaten exclusively are often toxic, whereas oak leaves eaten in addition to relatively small amounts of other feeds, such as hay, are much less toxic or nontoxic (49, 51). Another method of natural protection has been demonstrated in goats (41). The ruminal mucosae of goats have normal tannase activity which varies directly with the content of tannic acid of the rumen. This is thought to be a natural protective mechanism against tannic acid poisoning. Tannase activity has yet to be demonstrated in cattle. No direct relationship between the amount of oak leaves ingested and blood tannic acid levels has been demonstrated, indicating that the critical factor in oak poisoning is the amount of tannic acid absorbed, not the amount ingested (56). It has been suggested that the absorption of tannic acid is enhanced by the rumen stasis it causes. It was then postulated that the more resistant an animal is to the initial absorption of tannic acid, the less susceptible it is to tannic acid poisoning.

Oak poisoning can be prevented by use of calcium hydroxide as a feed additive. Rabbits given 1 part calcium hydroxide to 6 parts tannic acid were protected from poisoning (39). In separate experiments, calves were protected from oak poisoning by being fed a supplemental

ration which contained 9 and 10 percent calcium hydroxide (50, 51). Supplemental feeds which contained less than 9 percent calcium hydroxide were less effective in preventing oak poisoning, whereas feed containing greater than 10 percent calcium hydroxide became progressively less palatable.

There is no specific treatment for an animal once it has developed signs of oak poisoning (57). Orally administered mineral oil and fluids have been suggested as mild purgatives. General supportive care to correct dehydration and either the constipation or diarrhea have also been suggested.

CHAPTER III

Pathomorphology of blackjack oak (Quercus marilandica) leaf toxicity in the rabbit

SUMMARY

Blackjack oak (Quercus marilandica) leaves were administered to rabbits via a gastrocutaneous cannula at dose rates of 30, 45, and 55 gm/kg/day for periods of time ranging from 1 to 23 days. Control animals received only water via the same route. Three of 5 low-dose, 3 of 6 moderate-dose, and 1 of 4 high-dose animals developed mild light microscopic renal lesions. Control rabbits had no renal lesions, and neither principals nor controls had hepatic lesions. Renal lesions were seen in widely scattered groups of superficial cortical tubules and consisted of dilation of the tubules and necrosis and attenuation of the tubular epithelium.

Oak-leaf poisoning of cattle, sheep, and horses is an infrequent, yet economically important, condition encountered in veterinary medicine (4, 5, 6, 7, 22, 23). Of the more than 60 species of oak in North America, a small number have been reported as toxic, though all should be considered potentially toxic (37, 50).

Research on oak-leaf poisoning has been hampered by the high cost of animals (cattle) and the large volume of leaves needed. The present

study was conducted to develop an inexpensive laboratory animal model of oak-leaf poisoning in cattle.

Materials and Methods

Animals--Twenty-four female New Zealand white rabbits, each weighing approximately 2 kg, had a gastrocutaneous cannula surgically implanted in the left flank under general anesthesia with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg). Each was allowed at least 10 days to recover from the surgery prior to use in the study. Rabbits were randomly assigned to 4 groups of 6 by use of a random number table. Control rabbits received 75 ml/kg/day of tap water which was split into three aliquots and administered throughout the day. Low-dose rabbits received 30 gm/kg/day of ground wet-weight leaves (33% dry matter) split into three aliquots. Moderate-dose rabbits received 45 gm/kg/day of ground wet-weight leaves split into three aliquots. High-dose rabbits received 55 gm/kg/day of ground wet-weight leaves split into 4 aliquots. Leaves were administered as a slurry via the gastrocutaneous cannula by use of a dose syringe. Pelleted rabbit food and water were offered free choice throughout the experiment. Rabbits found dead or *in extremis* were immediately necropsied. Liver and kidney slices were fixed in Carson's fixative, processed routinely, embedded in paraffin, sectioned to 6 um, and stained with hematoxylin and eosin (58).

Oak-leaf preparation--Immature (less than 2 weeks post-bud stage) blackjack oak (*Quercus marilandica*) leaves were collected, ground in an electric meat grinder, and frozen at -5^{o} C until use. Leaf tannin levels, determined by a modified bovine serum albumin precipitation assay, have been previously reported (59). Prior to administration,

ground leaves were thawed and soaked in sufficient water that, when thoroughly mixed in a blender, produced a thick slurry.

Results

Seven of the original 24 rabbits were removed from the study while it was in progress because of problems other than those associated with oak-leaf administration; problems included respiratory pasteurellosis and self-inflicted trauma to the cannula site. The number of days of treatment and the incidence of hepatic and renal lesions are given in Table 1. No significant gross lesions were seen at necropsy. Microscopic renal lesions were seen in 3 of 5 low-dose, 3 of 6 moderate-dose, and 1 of 4 high-dose animals. Widely scattered groups of superficial cortical tubules were dilated and lined by altered epithelium (Fig 1). This lesion varied in severity from mild to very mild. Affected epithelial cells varied from necrotic cells with deeply eosinophilic cytoplasm and pyknotic nuclei to flattened cells which had slightly basophilic cytoplasm (Fig 2). Tubular lumens contained detached dead cells and/or basophilic stringy to eosinophilic granular material. No hepatic lesions were seen.

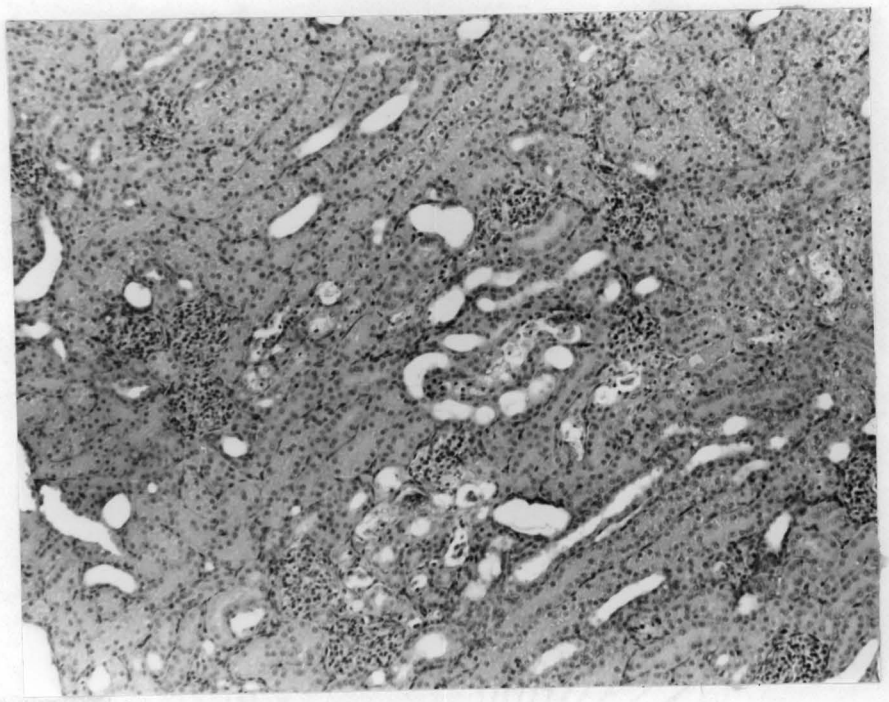
Discussion

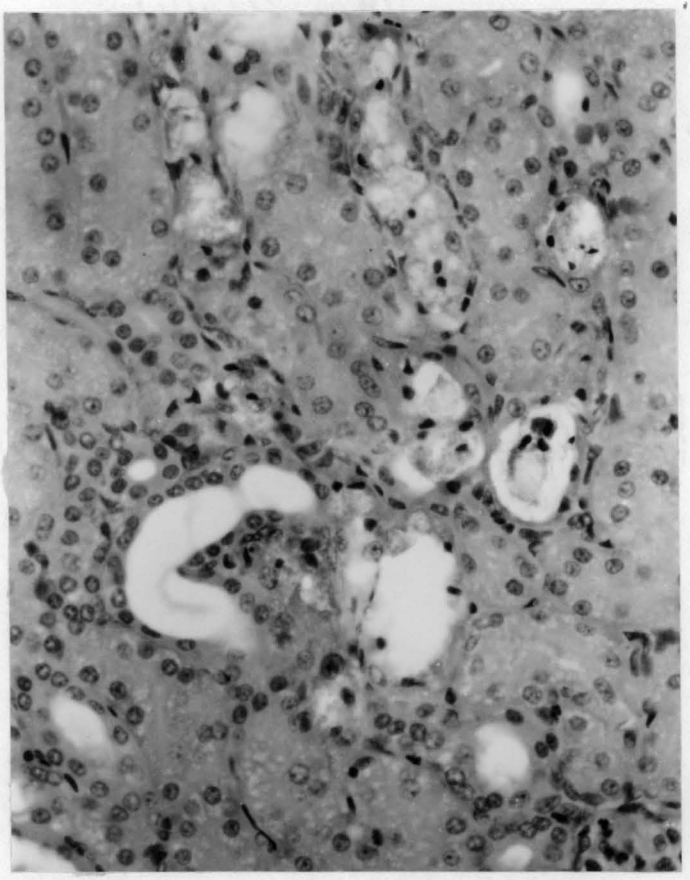
Forty-seven percent of the rabbits in the present study developed microscopic renal lesions in response to oak-leaf administration. There was, however, no clear pattern of incidence when dosage levels were compared. Although this study did not directly address why such variability occurred, some possible explanations must be considered. Individual variation by each rabbit in response to oak-leaf administra-

TABLE 1--Outcome of oak feeding trial in rabbits

Group	Rabbit	Days of Treatment	Microscopic Renal Lesions	Microscopic Hepatic Lesions
Control	2	9	Neg*	Neg
	5	3	Neg	Neg
Low Dose	6	23	Neg	Neg
	10	2	Mild	Neg
	11	23	Mild	Neg
	12	23	Mild	Neg
	20	3	Neg	Neg
Moderate Dose	13	1	Very Mild	Neg
	14	23	Neg	Neg
	15	2	Very Mild	Neg
	16	23	Neg	Neg
	17	8	Very Mild	Neg
	18	22	Neg	Neg
High Dose	1	2	Neg	Neg
	3	2	Neg	Neg
	4	2	Mild	Neg
	22	1	Neg	Neg

*Negative.





Fi -Renal lesion of oak leaf-poisoned rabbit demonstrating tubular di tion and epithelial cell necrosis. Hematoxylin and eosin (H&E) st X6.3.

Fi -Higher magnification of Fig 1. H&E stain; X25.

tion is a possible explanation for the variable occurrence of renal lesions. Variable responses to oak leaves in cattle have been described, though no explanation as to why such variation occurred was offered (49). It would not be unreasonable to expect a similar response in rabbits, particularly if progressive renal disease was not produced. No information is available to explain why the rabbit does not respond uniformly to the oak-leaf toxin, but possible explanations might include differences in bioactivation and/or absorption of the oak-leaf toxin, inadequate dose of toxin, or differences in rate of excretion. Another possible cause of variable incidence of renal lesions is that some other factor was altering the animals' response to oak leaves. Although no information is available to document the effects of stress, water loading, altered nutritional state, or the presence of a gastrocutaneous cannula on the development of oak-leaf poisoning in rabbits, these factors may have altered individual responses. It is known that simultaneous ingestion of oak leaves and normal feeds, such as hay, lessens the toxic effect of oak leaves in cattle (49, 51). A similar effect may have been seen in rabbits of this study because other feed was available throughout the test period. Finally, the possibility that renal lesions were caused by a factor other than oak-leaf toxin must be considered. Stress, water loading, altered nutritional state, the presence of a gastrocutaneous cannula, or some other unidentified factor possibly could have caused the renal lesions. The absence of lesions in control rabbits suggests that this is not the case. The number of control rabbits may not have been adequate to reflect the effects of some unknown factor, however.

Oak poisoning has been produced in rabbits by administration of a

crude tannin extract of shin oak (Quercus havardi) leaves (36). Lesions were reported as renal tubular necrosis and severe hepatic necrosis but were not fully described. While the previously reported study and the study reported herein cannot be compared directly because of differences in species of oak, material administered, and dose rate, it is interesting to note that a similar renal lesion was produced. The absence of a hepatic lesion in rabbits of the present study may reflect the differences previously mentioned between the 2 studies.

In a previously reported study, rabbits offered Quercus marilandica leaves free choice did not develop signs or lesions of oak-leaf toxicity (37). The findings of the present study differ from those of that study and probably reflect differences in experimental design. It is likely that rabbits in the present study received a higher dose of leaves than did those of the previous study. Differences in the toxin content of leaves is also a possible explanation for the discrepancy between the 2 studies.

Renal lesions in rabbits of the present study were similar to, though much less severe, than those described in oak-poisoned cattle (6, 17, 22, 24). The mild nature of the renal lesion in rabbits of this study suggests that the combination of procedure, materials, and animals used in this study produced a model which is in need of further refinement to be of value in future study of oak-leaf poisoning.

CHAPTER IV

Pathomorphology of blackjack oak (*Quercus marilandica*) leaf toxicity in the mouse

SUMMARY

Blackjack oak (*Quercus marilandica*) leaves were offered to mice free choice in a crumble-type ration which contained 25, 50, and 100% leaves. Control mice were offered a similar crumble-type ration which contained no leaves. All mice were euthanatized after 14 days, and none had renal or hepatic lesions of oak-leaf toxicity. The mouse does not appear to be susceptible to poisoning by blackjack oak leaves at the dosage level and under conditions of the present experiment.

The mouse has been used as a bioassay tool for toxicity of various components of acorns (45). The route of administration of the acorn components and the omission of pathology data make the results of that report suspect. The present study was conducted to better define the potential of the mouse as a model of oak-leaf poisoning.

Materials and Methods

Animals--Twenty-eight mice were randomly assigned to 4 groups of 7 mice each by use of a random number table. Prior to the study, the mice received pelleted mouse feed and water free choice. At the onset of the

study, mouse pellets were replaced by the appropriate oak-leaf ration. All mice were euthanatized by cervical dislocation after 14 days of treatment and were necropsied immediately. Kidney and liver sections were fixed in Carson's fixative, processed routinely, embedded in paraffin, cut to 6 um, and stained with hematoxylin and eosin (58).

Ration preparation--The collection, preparation, and tannin assay of blackjack oak (Quercus marilandica) leaves used in this study have been previously described (59). Ground oak leaves were mixed with ground mouse feed pellets and molasses (150 ml/kg feed) in proportions to produce rations which contained 0, 25, 50, and 100% oak leaves, spread in trays to dry, manually broken into a crumble-type feed, and frozen at -5^{o} C until use.

Results

There were no oak poisoning-related deaths in any group of mice. Mice consuming rations containing 50 and 100% oak leaves had moderate to severe serous atrophy of body fat. Microscopically, no animal had renal or hepatic lesions attributable to oak poisoning.

Discussion

In a previous study, it was reported that a tannin isolated from acorns, when administered intraperitoneally to mice, caused death (45). Similar injections of other acorn components such as gallic acid, quercitol, quercitrin, and an unidentified alkaloid into mice had no effect. The pathogenesis of the deaths of these mice was not investigated by clinicopathologic or morphologic means. The lack of such data prevents full interpretation of the results. In the present study, mice were not

killed by ingestion of blackjack oak leaves and did not develop renal or hepatic lesions. This suggests that such leaves are not toxic to mice when ingested at the rates of consumption seen in this experiment. The possibility still exists that another species or source of oak leaves or direct oral administration of isolated components of oak leaves might be toxic to mice.

CHAPTER V

An investigation of the suitability of sheep as a model of blackjack oak (Quercus marilandica) leaf toxicity in cattle

SUMMARY

Three sheep were given blackjack oak (Quercus marilandica) leaves via a ruminal cannula at an average daily dose of 1.32% BW of wet-weight leaves (33% DW) for periods ranging from 3 to 21 days. All sheep developed renal tubular epithelial degeneration and necrosis of varying degrees of severity. Sheep appear to be susceptible to oak-leaf poisoning and may have potential for use in investigation of this condition.

Sheep have been reported to be susceptible to natural poisoning by leaves and buds of common shin oak (Quercus breviloba) and to experimental poisoning by acorns of common shin oak and leaves of sand shin oak (Quercus havardi) (5, 9, 37). The gross and histologic lesions in sheep have been reported to be similar to those in cattle. The present study was conducted to identify a toxic dose of blackjack oak (Quercus marilandica) leaves in sheep and to identify target organs of oak toxicity.

Materials and Methods

Animals--Three mixed-breed, 6-month-old, female sheep were anesthetized with intramuscular xylazine (1 mg/kg), and a ruminal cannula was surgically placed in the left flank. Each sheep received 70,000 units/kg of procaine penicillin G per day for 5 days following surgery and was allowed at least 7 days to recover before the start of the experiment. Alfalfa hay, 14% protein concentrate, and water were available free choice.

Sheep were assigned a dose of 2, 3, or 5% of body weight of wet-weight blackjack oak (Quercus marilandica) leaves per day by use of a random number table. Leaves were administered every day unless ruminal impaction was observed. Sheep which had ruminal impaction received water and/or mineral oil until the condition was corrected, at which time leaf administration was resumed. Mechanically ground oak leaves were administered via the ruminal cannula, and approximately 1 liter of water was also given to aid in the prevention of ruminal impaction. Sheep found dead or in extremis were necropsied immediately. Samples of brain, lung, heart, liver, kidney, spleen, mesenteric lymph node, urinary bladder, skeletal muscle, sciatic nerve, uterus, ovary, adrenal gland, thyroid, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, spiral colon, and cecum were fixed in Carson's fixative, routinely processed, embedded in paraffin, cut to 6 um, and stained with hematoxylin and eosin (58).

Oak-leaf preparation--The collection, preparation, and tannin assay of blackjack oak leaves used in this study has been described (59). Leaves were stored frozen at -5° C and thawed at 25° C immediately prior

to administration.

Results

Full dose rates of oak-leaf administration were not attained in this study due to the frequent occurrence of ruminal impaction. Doses achieved are shown in Table 2.

Gross lesions were not seen in any animal. All sheep had microscopic renal lesions consisting of small- to moderate-sized groups of cortical tubules which had mild to moderate tubular epithelial cell degeneration and necrosis. Degenerating tubules were lined by epithelial cells which contained hyaline droplets in their apical cytoplasm. Necrotic tubules were lined by epithelial cells which had deeply eosinophilic cytoplasm and pyknotic nuclei and which often contained pink homogeneous to eosinophilic granular material. Many tubules were dilated and lined by flattened epithelial cells. All other tissues examined microscopically were normal.

Discussion

Ruminal impaction prevented the administration of the planned doses of oak leaves to sheep in this study. Ruminal impaction may have been a result of the sudden addition of a mass of coarse material into the rumen or an effect of the leaf toxin or both. The mean value of the average daily dose of wet-weight leaves administered to sheep was 1.32% BW. This value coupled with the occurrence of lesions in all 3 sheep suggests that a dose of 1.5% BW per day would lead to a reasonable incidence of renal lesions and might be less likely to cause ruminal impaction.

TABLE 2--Oak leaf-administration schedule in sheep

Sheep	Total Dose of Leaves Given (kg)	Duration of Leaf Administration (Days)	Average Daily Dose of Wet-Weight Oak Leaves (% BW)
1	2.9	10	1.52
2	0.35	3	1.00
4	6.6	21	1.45
			*$\bar{x}$ = 1.32

*Mean.

The renal lesion that developed in sheep of the present study resembles the lesion resulting from poisoning of sheep by acorns of Quercus lobata (9). The presence of such a lesion suggests that sheep may be a useful model in which to study oak-leaf poisoning.

CHAPTER VI

Renal clearance studies in sheep given blackjack oak (*Quercus marilandica*) leaves

SUMMARY

Five sheep were given blackjack oak (*Quercus marilandica*) leaves via a ruminal cannula at a dose of 1.5% BW of wet-weight leaves (33% DW) for periods of time varying from 8 to 22 days. Renal clearances of inulin and para-aminohippuric acid were performed prior to and during the period of oak-leaf administration. In addition, each animal received serial clinicopathologic examinations, was necropsied, and tissues were examined microscopically. One sheep had decreases in glomerular filtration rate (GFR) and total renal blood flow (TRBF), clinicopathologic changes of renal failure, and microscopic renal lesions of oak toxicity. A second sheep had similar, though somewhat variable, changes in GFR and TRBF, similar renal lesions, but lacked clinicopathologic changes. A third sheep had very mild microscopic lesions only, and a fourth sheep had no changes in any parameter. A fifth sheep had increases in clearance values, no changes in clinical pathology, and no renal lesions. The results of this study suggest that oak-leaf toxicity may produce similar changes in GFR and TRBF to that of models of acute toxic and ischemic renal tubular necrosis but that

interpretation of the results remains speculative.

Oak-leaf and acorn toxicity in cattle, sheep, and horses is an infrequent, yet potentially economically devastating, condition seen in veterinary medicine (4, 5, 6, 7, 17, 22, 23). While the occurrence of this condition is well documented, little has been done to elucidate the pathogenesis of the major microscopic lesion, renal tubular necrosis.

Common features of models of ischemic and toxic renal tubular necrosis include decreases in total renal blood flow (TRBF) and glomerular filtration rate (GFR) (60, 61). The causal relationship of these changes to the primary pathogenetic event is not known. Only 1 very limited study of renal clearance function in oak-poisoned animals has been performed (9). This study demonstrated a decrease in clearance of sodium sulfanilate in 1 oak-poisoned animal. The present study was performed to determine the effect of oak-leaf administration on TRBF and GFR in sheep. In addition, an attempt was made to identify a chronologic relationship between changes in renal blood flow and the occurrence of tubular necrosis.

Materials and Methods

Oak-leaf collection and handling--Immature (less than 2 weeks post-bud stage) blackjack oak (Quercus marilandica) leaves were collected in early spring, coarsely ground with an electric meat grinder, thoroughly mixed, and frozen at -5° C until use. Tannin levels of the leaves were determined using a modification of a previously described method (method 1) (62). Briefly, a stock leaf extract was produced by extraction of

dried leaves with boiling 50% (v/v) aqueous methanol. Aliquots of the stock extract were reacted with a standard amount of bovine serum albumin (BSA), the precipitated BSA centrifuged out, and the supernatant assayed for the remaining BSA. The BSA content of the supernatant was determined by use of a Bio Rad Protein Assay but with replacement of the suggested gel filtration step by extract/reagent controls. The sample absorbance at 595 um was compared to a standard BSA curve. The results of this test were then compared to those for other species of oak trees (62).

In addition, a comparison of the ability to precipitate BSA by leaf extract and technical-grade tannic acid was performed (method 2). Briefly, a standard curve was prepared by reacting a standard amount of BSA (1.4 mg) with varying amounts of tannic acid (0.02-0.1 mg). After precipitation, the precipitated BSA was centrifuged out and the supernatant assayed for the remaining BSA. The BSA content of the supernatant was determined by use of a Bio Rad Protein Assay. Next, an aliquot of leaf extract was reacted with the standard amount of BSA, and the BSA content of the supernatant was determined. By comparison of the extract precipitation with the standard curve, a tannic acid equivalent for the leaf extract could be determined.

Animals--Five 6-month-old, mixed-breed, female sheep were anesthetized with intramuscular xylazine (1 mg/kg), and a ruminal cannula was surgically implanted in the left flank. Each sheep received 70,000 units/kg of procaine penicillin G per day for 5 days following surgery and was allowed at least 7 days to recover before the start of the experiment.

Oak leaves were administered via ruminal cannula once per day at a

dose rate of 1.5% BW of wet-weight leaves. Approximately 1 liter of water was given with the leaves in an attempt to prevent ruminal impaction. Alfalfa hay, 14% protein concentrate, and water were offered free choice.

Renal clearance studies--The inulin clearance test is a measure of glomerular filtration rate (GFR), whereas the clearance of PAH is a measure of total renal blood flow (TRBF). Renal clearances of inulin and para-aminohippuric acid (PAH) were performed twice prior to oak-leaf administration and from 3 to 7 times during administration. Leaf administration was started the day following the second pretreatment clearance study, and clearance studies were performed every third day throughout the period of leaf administration. Clearance techniques used in this study were adapted from published techniques (63, 64).

Sheep were restrained in stocks, bilateral jugular catheters and a urinary catheter were placed, baseline blood and urine samples were taken, and loading doses of I.V. fluids and clearance materials were given. Intravenous normal saline was given initially at a dose of 40 ml/kg over 45 minutes to ensure adequate urine flow. Loading doses of inulin (50 mg/kg) and PAH (8 mg/kg) were given at the onset to rapidly produce appropriate blood levels. Immediately following the loading dose of clearance materials, a sustaining infusion was started to maintain appropriate blood levels. Clearance materials were prepared in normal saline in concentrations to maintain blood levels of 0.25 mg/ml inulin and 0.02 mg/ml PAH at I.V. drip rates of 2 ml/minute.

Following the 45-minute equilibration period, 6 consecutive 20-minute test periods were begun. Urine was collected throughout each period, and a blood sample was taken at the midpoint.

Inulin concentrations in serum and urine were measured spectrophotometrically by a method based on the reaction of inulin and indole-3-acetic acid (65). PAH concentrations in serum and urine were measured photometrically by the reaction of PAH and N-(1-naphthyl) ethylenediamine (63). Clearance values were calculated from blood and urine concentrations of each clearance material. Control clearance values were averaged and compared to each set of treatment clearance values. Examination for significant changes in clearance values was carried out by use of a two-tailed Student's t test (66). Differences between clearance values were considered significant if $p < 0.05$.

Pathology--Blood and urine samples were taken presurgically, prior to each clearance study, and prior to euthanasia when possible. A complete blood count was performed on EDTA blood. Serum chemistries included albumin, total bilirubin, blood urea nitrogen, calcium, chloride, creatinine, glucose, sodium, potassium, osmolality, phosphorus, and serum glutamic pyruvic transaminase. Urine chemistries included specific gravity, creatinine, sodium, and osmolality. Calculations included urine creatinine/plasma creatinine, urine osmolality/ plasma osmolality, and fractional sodium excretion [Equation (1)].

$$FE_{Na} = \frac{[Cr]p}{[Cr]u} \times \frac{[Na]u}{[Na]p} \times 100 \qquad (1)$$

Animals found dead, in extremis, or which had developed problems unassociated with oak-leaf toxicity were necropsied immediately. Sections of brain, lung, heart, liver, kidney, spleen, mesenteric lymph node, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, spiral colon, and cecum were collected for microscopic examination.

Tissues were fixed in Carson's fixative, processed routinely, embedded in paraffin, cut to 6 um, and stained with hematoxylin and eosin (58).

Results

Leaves used in this study contained 33% solids on a dry-weight (DW) basis. Tannin levels of leaf extracts determined by method 1 are reported as mg BSA precipitated per mg of leaf. Oak leaves used in this experiment had a BSA precipitation value of 0.344 mg BSA pptd/mg leaf. Published BSA precipitation values for other species of oak leaves vary from 0.091 to 0.326 mg BSA pptd/mg leaf (62). Tannin levels of leaf extracts determined by method 2 are reported as a comparison of the ability of 1 mg of leaf to precipitate BSA when compared to tannic acid. One milligram of dried oak leaves used in this study precipitated the same amount of BSA as did 0.033 mg of tannic acid. Stated another way, the leaves used in this study contained the equivalent of 3.3% tannic acid (DW).

Clearance study data, clinical pathology data, and necropsy results will be considered separately for each sheep. Tables 3 and 4 in the text contain a compilation of clearance study data and calculations, and Tables 7, 8, 9, and 10 in Appendix A contain a compilation of clinical pathology data and calculations for all sheep in this experiment.

Pretreatment GFR and TRBF mean values of sheep 6 were similar to those reported for normal sheep (64). Normal mean values in sheep for GFR are 2.59 ml/min/kg and for TRBF are 18.17 ml/min/kg (64). These reported values did not include a test for significant difference between animals. Sheep 6 of the present experiment had statistically significant differences between GFR and TRBF values of pretreatment

TABLE 3--Glomerular filtration rate (GFR) and total renal blood flow (TRBF) values of oak leaf-treated sheep

Sheep	Number of Days of Intraruminal Oak Leaf Administration									C^{o}
	Cont 1+	Cont 2+	2	5	8	11	14	17	20	
GFR										
6	2.57*	1.83	0.63	0.78	0.54					2.20
7	2.35	2.21	3.33	1.77	1.98	1.98	2.07	1.98	2.17	2.29
8	2.19	3.17	3.40	3.08	3.20	3.48				2.72
11	1.51	1.19	1.99	1.65	1.68					1.35
13	1.81	1.83	1.80	2.48	1.42					1.82
TRBF										
6	25.52	15.88	5.88	13.79	3.19					20.70
7	19.34	13.90	13.88	12.37	18.93	11.45	12.18	13.15	18.05	16.87
8	14.31	16.66	15.99	13.57	20.08	21.54				15.59
11	10.24	16.90	17.23	8.87	9.65					13.57
13	12.10	17.76	11.09	14.86	9.03					14.93

*All values reported as ml/min/kg and are means of the individual clearance intervals.
+Control numbers 1 and 2 are pretreatment clearance studies.
^{o}Mean value of pretreatment clearance studies 1 and 2.

TABLE 4--Statistical comparison of glomerular filtration rate (GFR) and total renal blood flow (TRBF) values in oak leaf-treated sheep

Sheep	Control 1 vs 2	C+ vs Day 2	C vs Day 5	C vs Day 8	C vs Day 11	C vs Day 14	C vs Day 17	C vs Day 20
GFR								
6	< 0.001*	< 0.001	< 0.001	< 0.001				
7	< 0.5	< 0.001	< 0.005	< 0.05	< 0.05	< 0.2	< 0.05	< 0.4
8	< 0.001	< 0.025	< 0.4	< 0.1	< 0.01			
11	< 0.2	< 0.005	< 0.2	< 0.1				
13	> 0.5	> 0.5	< 0.2	< 0.1				
TRBF								
6	< 0.001	< 0.001	< 0.025	< 0.001				
7	< 0.025	< 0.2	< 0.025	< 0.4	< 0.01	< 0.05	< 0.1	> 0.5
8	< 0.025	> 0.5	< 0.4	< 0.001	< 0.001			
11	< 0.05	< 0.2	< 0.1	< 0.1				
13	< 0.025	< 0.1	> 0.5	< 0.025				

*Data reported as p values.
+Mean value of pretreatment clearance studies 1 and 2.

clearance studies 1 and 2. All 12 clearance period values from pretreatment clearance studies 1 and 2 were combined in a mean value for GFR or TRBF for use in comparison with treatment clearance values. Statistically significant decreases in GFR and TRBF were seen between pretreatment mean values and those of days 2, 5, and 8 of oak-leaf administration.

Sheep 6 had an increased white blood cell count (WBC) with neutrophilia and lymphopenia. Increases in blood urea nitrogen (BUN) and serum creatinine, phosphorus, total bilirubin, and serum glutamic pyruvic transaminase (SGPT) were seen as well as decreases in serum sodium and potassium. Chloride values were erratic, and all other blood and serum values were normal. Urine osmolality and creatinine values were very erratic, sodium was low, and specific gravity was decreased.

Gross lesions of sheep 6 consisted of widespread serous atrophy of body fat and skin necrosis and purulent cellulitis of the muscle fascia and subcutis adjacent to the ruminal cannula. Microscopic lesions were seen only in kidney and liver. Microscopic renal lesions consisted of moderately severe tubular epithelial degeneration and necrosis of groups of proximal convoluted and straight tubules. Microscopic hepatic lesions consisted of severe diffuse hepatocytic vacuolation and cytoplasmic rarefaction and individual hepatocellular death.

Pretreatment GFR and TRBF mean values of sheep 7 were similar to those reported for normal sheep (64). No statistically significant difference in GFR was seen between pretreatment clearance studies 1 and 2. There was, however, a statistically significant difference in TRBF between pretreatment clearance studies 1 and 2. Glomerular filtration rate was decreased significantly on days 5, 8, 11, and 17, increased

significantly on day 2, and was not significantly changed on days 14 and 20 of oak-leaf administration. Total renal blood flow was decreased significantly on days 5, 11, and 14 and had no significant change on days 2, 8, 17, and 20 of oak-leaf administration.

Sheep 7 had decreases in hematocrit and hemoglobin (days 5 to 20), albumin (days 8 to 20), and calcium (days 17 to 20). Phosphorus and osmolality values were erratic. Hemoglobinemia was evident in serum samples. All other blood and serum values were normal. Urine osmolality and creatinine values were very erratic, and urine sodium was low. Urine specific gravity (SG) was low on day 20 of oak-leaf administration.

The only gross lesion seen in sheep 7 was widespread serous atrophy of fat. Microscopic lesions were seen only in the kidney and consisted of widely separated groups of cortical tubules which were dilated and were lined by flattened epithelium.

Pretreatment GFR and TRBF mean values of sheep 8 were similar to those reported for normal sheep (64). Sheep 8 had statistically significant differences between GFR and TRBF values of pretreatment clearance studies 1 and 2. Glomerular filtration rate was increased significantly on days 2 and 11 and had no significant changes on days 5 and 8 of oak-leaf administration. Total renal blood flow had no significant changes on days 2 and 5 and was increased significantly on days 8 and 11 of oak-leaf administration.

Sheep 8 had a gradually increasing WBC with a normal differential. The BUN was increased prior to ruminal cannula implantation but decreased when clearance studies were begun. Urine SG, creatinine, sodium, and osmolality values were very erratic.

Gross lesions in sheep 8 consisted of widespread serous atrophy of fat, and skin necrosis and purulent cellulitis of the muscle fascia and subcutis adjacent to the ruminal cannula. No microscopic lesions were seen in any tissue.

Pretreatment GFR and TRBF mean values of sheep 11 were not similar to those reported for normal sheep (64). Sheep 11 had no statistically significant difference between GFR values of pretreatment clearance studies 1 and 2. There was, however, a statistically significant difference in TRBF between pretreatment clearance studies 1 and 2. Glomerular filtration rate was increased significantly on day 2 and had no significant change on days 5 and 8 of oak-leaf administration. Total renal blood flow had no significant change on days 2, 5, and 8 of oak-leaf administration.

Sheep 11 had a transient increase in WBC between days 5 and 8 of oak-leaf administration. A decrease in hematocrit and hemoglobin and occurrence of hemoglobinemia were also seen. All other blood and serum values were normal. Urine SG was decreased on days 5 and 8. Urine creatinine, sodium, and osmolality values were very erratic.

The only gross lesion seen in sheep 11 was widespread serous atrophy of fat. Only renal lesions were seen microscopically and consisted of widely scattered groups of cortical tubules which were dilated and lined by flattened epithelium.

Pretreatment GFR and TRBF mean values of sheep 13 were not similar to those reported for normal sheep (64). No significant difference in GFR was seen between pretreatment clearances 1 and 2, and no significant changes in GFR were seen on days 2, 5, and 8 of oak-leaf administration. A statistically significant difference in TRBF was seen between pre-

treatment clearances 1 and 2. A significant decrease in TRBF was seen on day 8, and no significant changes were seen on days 2 and 5 of oakleaf administration.

Sheep 13 had a transiently increased WBC with neutrophilia at the time of pretreatment clearance 2. All other blood and serum chemistry values were normal. Urine SG was decreased on day 8. Urine creatinine, sodium, and osmolality values were very erratic.

The only gross lesion seen in sheep 13 was widespread serous atrophy of fat. No microscopic lesions were seen in any tissue.

Discussion

Pretreatment clearance GFR and TRBF values of sheep 6, 7, and 8 were similar to those previously reported in sheep, whereas those of sheep 11 and 13 were not. A small amount of variation between animals would be expected, but GFR and TRBF values of sheep 11 and 13 were considerably lower than published values. Clearance studies were performed uniformly in all animals so that any variation in GFR and TRBF would most likely be due to variation in individual animal's response to the studies. Factors which may have affected the response of sheep in this experiment include postsurgical alterations in feed and water intake, response to the stress of daily manipulation, and alterations in the ability to rid themselves of fluids given during clearance studies.

Statistically significant differences in GFR were seen between pretreatment clearance studies 1 and 2 in sheep 6 and 8 and in TRBF in all sheep. Variability in pretreatment data may be due to several possible causes. Because relatively small numbers of control values were used in determination of significant differences, such differences

may reflect the small sample size rather than any real differences in clearance ability between control clearances. Other factors may have caused true alterations in GFR and TRBF between pretreatment clearance studies. The animals' response to the stress of handling and possible variations in state of hydration and nutrition may have truly altered GFR and TRBF. Published normal values of GFR and TRBF in sheep did not include a test for significance between animals, so no conclusions about normal individual animal variation can be drawn based on that data (64).

Sheep 6 had statistically significant decreases in GFR and TRBF associated with oak-leaf administration. The decreases in GFR and TRBF were similar to those seen in models of acute toxic and acute ischemic renal failure in other species (60, 61). Decreases in GFR and TRBF in reported models of toxic and ischemic renal failure and in the current study were not determined to be primary or secondary in nature. This failure of distinction results from the similar effects of ischemic and toxic mechanisms on GFR and TRBF. In experimental ischemic renal failure, a vasoconstricting drug, such as norepinephrine, is the primary factor in the pathogenetic sequence. Decreases in TRBF followed by decreases in GFR occur and are followed closely by ischemic tubular necrosis. Subsequently, interstitial edema is seen. Tubular compression by the expanded interstitium and obstruction by detached epithelial cells occur. Tubular obstruction causes increased intratubular pressure and a retrograde increase in pressure in Bowman's space. This increased pressure causes a further decrease in GFR and leads to azotemia. In experimental ischemic renal failure, a clear relationship between an ischemic insult and a decrease in TRBF can be seen. In acute experimental toxic renal failure, the toxin causes direct tubular damage which

results in altered sodium reabsorption and subsequent activation of the renin-angiotensin system. Increased renal cortical vascular resistance follows and causes decreased cortical blood flow. Cortical ischemia leads to further renal tubular damage. It can be seen that decreases in total renal blood flow and glomerular filtration rate play a role in both ischemic and toxic renal failure. Therefore, it has been demonstrated that sheep 6 had similar pathophysiologic changes to both toxic and ischemic models of renal failure and that a specific pathogenetic sequence cannot be proposed based on GFR and TRBF changes alone.

An attempt was made in all sheep to determine whether or not tubular damage preceded changes in GFR and TRBF by use of the fractional sodium excretion test, but the results of this test were very erratic and were not suitable for interpretation. The results of urine creatinine/plasma creatinine and urine osmolality/plasma osmolality were also very erratic.

Sheep 7 had erratic changes in GFR and TRBF during oak-leaf administration. The erratic nature of the changes is not easily explained. It is possible that the GFR and TRBF of this animal varied considerably from day to day as a normal occurrence. Alternatively, effects of the oak-leaf toxin may have varied due to variations in bioactivation, absorption, and excretion of the toxin. Finally, the ability of the sheep to rid itself of the water load provided by leaf administration and the clearance studies may have altered the animal's response to the leaf toxin or the clearance studies themselves.

Sheep 8 had statistically significant increases in GFR and TRBF during oak-leaf administration. Also seen were increased BUN and a normal serum creatinine presurgically, possibly indicating prerenal

azotemia. A possible explanation for the decrease in BUN and improvement of GFR and TRBF during the clearance studies is that the fluids given as part of the clearance studies corrected the prerenal azotemia and improved the health of the animal. No deleterious effect of the oak-leaf administration was seen. This, too, would allow an improvement in the condition of the sheep.

Sheep 11 and 13 had little or no significant change in GFR and TRBF associated with oak-leaf administration. It would appear that these animals were not susceptible to the oak leaves used in this study. The administration of intravenous fluids as part of the clearance studies may have exerted a protective effect on these 2 sheep. In both the ischemic and toxic models of renal failure, a decrease in cortical blood flow is part of the pathogenesis. It is possible that the expansion of the blood volume produced by I.V. fluids created a protective effect against part of the mechanism of renal lesion development.

Sheep 6 had an increased WBC which was most likely a response to the necrosis and infection associated with the ruminal cannula. Increases of BUN, creatinine, and phosphorus were strongly indicative of renal disease. This was confirmed by the presence of kidney lesions. The renal lesions were similar to those described for oak toxicity in sheep (9). Severe hepatic degeneration and necrosis were also seen in sheep 6 in addition to increases in total bilirubin and SGOT. Hepatic lesions are not commonly reported in oak-poisoned animals. Hepatic lesions may have been due to an effect of the oak toxin or some effect of the clearance studies, but no indication of the cause was seen. Decreases in serum sodium and potassium and in urine SG may have been due to excessive fluid administration or to renal disease. No clinico-

pathologic evidence of overhydration was seen, however, so the cause of these changes was most likely renal disease. Erratic urine creatinine, osmolality, and sodium values were seen in sheep 6 and in all other sheep. A variety of factors may have contributed to the lack of consistency, including decreased salt intake because the sheep refused any form of concentrate ration, high urine flow due to large volumes of I.V. and intraruminal fluids, and effects of the clearance materials themselves.

Sheep 7 had a decreased hematocrit and hemoglobin. The anemia was possibly the result of intravascular hemolysis which was reflected by the hemoglobinemia. It is possible that either the clearance materials themselves or the osmotic effects of the I.V. fluids may have caused the hemolysis. Intravascular hemolysis has not been described with oak poisoning. The decrease in albumin and urine SG were possibly due to dilution by the large volume of fluids given to the animal. The light microscopic renal lesion was so mild that it is unlikely that the low albumin and urine SG were the result of renal dysfunction.

Sheep 8 had an increased WBC which was probably a response to the necrosis and infection associated with the ruminal cannula.

The cause of the transient increase in WBC of sheep 11 on days 5-8 is unknown. The anemia seen in sheep 11 may have been caused by the same circumstances as those of sheep 7. The decreased urine SG may have been a result of fluid administration to the animal.

The cause of the transient increase in WBC of sheep 13 at the time of pretreatment clearance study 2 is unknown. The decrease of urine SG may have been the result of fluid administration to the animal.

The changes in sheep 6 suggest that decreases in TRBF and GFR play

a role in the pathogenesis of oak-leaf poisoning in sheep. With the exception of sheep 6, no clear correlation of clearance clinical pathology data and microscopic findings can be seen (Table 5). The low incidence of clearance changes in the sheep of this experiment makes it difficult to offer any firm interpretation of the data.

TABLE 5--Comparison of glomerular filtration rate (GFR) and total renal blood flow (TRBF) changes, clinical pathology data, and microscopic findings in oak leaf-treated sheep

Sheep	Statistically Significant Clearance Changes		Clinical Pathology Consistent with Renal Failure and/or Hepatic Disease	Kidney Lesions	Liver Lesions
	GFR	TRBF			
6	+	+	Renal/Hepatic	Moderate	Severe
7	Variable		Neg	Very Mild	Neg
8	Variable		Neg	Neg	Neg
11	Neg*	Neg	Neg	Very Mild	Neg
13	Neg	Neg	Neg	Neg	Neg

*Negative.

CHAPTER VII

A serial morphologic study of the renal lesions of oak (Quercus marilandica) leaf poisoned sheep

SUMMARY

Five of 7 sheep given blackjack oak (Quercus marilandica) leaves developed renal lesions of oak-leaf toxicity within 5 days of treatment. Sheep were given oak leaves via a ruminal cannula and were subjected to serial renal biopsies. Biopsy tissues were examined by light and electron microscopy. Light microscopy revealed multifocal renal cortical tubular epithelial degeneration and necrosis. Ribosomal disaggregation from rough endoplasmic reticulum, polyribosome disaggregation, cellular swelling, detachment, and death were seen by electron microscopy and suggest that the renal lesion of oak-leaf toxicity is a result of direct tubular epithelial cell toxicity.

Clinical reports of poisoning of cattle, sheep, and horses by oak (Quercus spp.) leaves and acorns are not uncommon in veterinary medical literature. Many reports do not, however, include information regarding microscopic examination of affected tissues; none have included ultrastructural findings. Thus, there is a need for such information.

Because sheep poisoned by blackjack oak (Quercus marilandica)

leaves and other published experimental models of acute renal disease have similar pathophysiologic and clinical pathologic changes, a morphologic comparison of these models is potentially beneficial (60, 61). The purpose of this study was to document the progression of the renal lesion of oak-leaf toxicity in sheep in hopes that this information would aid in the development of improved preventative and therapeutic measures.

Materials and Methods

Animals--Seven mixed-breed, 1-year-old, female sheep had ruminal cannulas surgically implanted by a previously described protocol (59). Each sheep was subjected to a pretreatment renal biopsy 10 days after cannula implantation, and pretreatment clinical pathology samples were taken. Oak-leaf administration was begun the day following the pretreatment renal biopsy at a dose of 1.5% BW of wet-weight leaves (0.495 g tannic acid/kg BW). Leaf preparation and tannin assay have been previously described (59). The first biopsy after the initiation of treatment was taken on day 2, and a biopsy was taken every third day thereafter until the animal was euthanatized. Sheep found *in extremis* or with problems unassociated with oak-leaf toxicity were euthanatized with an intravenous euthanasia solution[a] and immediately necropsied. Clinical pathology samples were taken prior to each biopsy and euthanasia.

Biopsy procedure--Sheep were anesthetized with intramuscular xyla-

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[a]T-61 Euthanasia Solution, American Hoechst Corporation, New Jersey.

zine (1 mg/kg) and received a paravertebral nerve block on the right side of vertebrae T_{13}, L_1, and L_2 with 2% lidocaine hydrochloride (67). The area was clipped, scrubbed, and an incision made 2 cm posterior to the last rib and 4 cm ventral to the lateral processes of the lumbar vertebrae. The kidney was exposed, and a sample was taken using a biopsy needle.[b] All body wall layers and skin were closed with medium polyamide nonabsorbable suture. The biopsy sample was split between vials of Carson's fixative for light microscopic examination and Karnovsky's fixative for electron microscopic examination (58, 68).

Pathologic examinations--Complete blood counts were performed on EDTA blood. Serum chemistries performed included albumin, total bilirubin, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, sodium, potassium, osmolality, phosphorus, and serum glutamic oxaloacetic transaminase (SGOT).

All sheep were necropsied immediately after euthanasia. Samples of brain, lung, heart, liver, kidney, spleen, mesenteric lymph node, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, spiral colon, and cecum were collected. Tissues were fixed in Carson's fixative, routinely processed, embedded in paraffin, sectioned to 6 um, and stained with hematoxylin and eosin. Kidney biopsy samples were handled similarly. Tissues for electron microscopy were fixed in Karnovsky's fixative, post-fixed in osmium tetroxide, embedded in epoxy resin,[c] stained in block with uranyl acetate, thin-sectioned, and stained on the grid

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[b]Tru-Cut Disposable Biopsy Needle, Travenol Laboratories, Deerfield, Illinois.

[c]Poly/Bed 812, Polysciences, Ltd., Warrington, Pennsylvania.

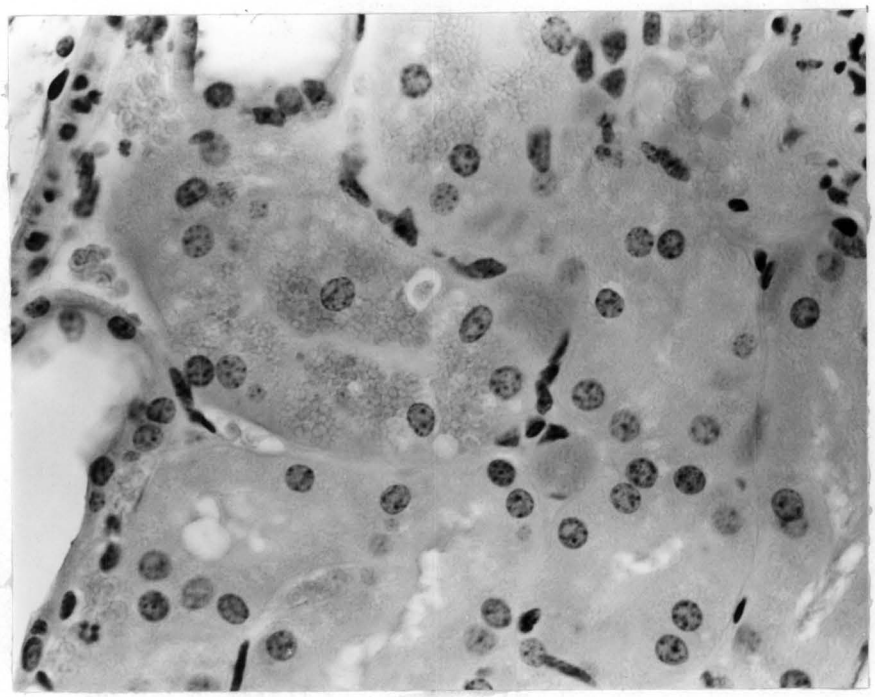
with uranyl acetate and lead citrate. Thick sections of 5 blocks of tissue were examined for each biopsy samples.

Results

Changes in clinicopathologic parameters measured in this experiment were not consistent amongst sheep. A compilation of clinical pathology data is presented in Tables 11 and 12 of Appendix B. Five sheep (Nos. 5, 14, 15, 16, 17) had decreases in serum sodium, 6 (Nos. 5, 14, 15, 16, 17, 18) had decreases in serum potassium, and 2 (Nos. 5, 16) had decreases in serum chloride, and 1 (No. 15) had an increased SGOT. Four sheep (Nos. 5, 16, 18, 19) had increases in WBC with all 4 having neutrophilia, 3 (Nos. 5, 16, 18) having lymphopenia, and 1 (No. 5) with a left shift. All sheep had very erratic and generally elevated serum glucose levels. In addition to previously mentioned changes, sheep No. 5 had increases of hematocrit, serum protein, BUN, creatinine, total bilirubin, and SGOT.

Grossly, sheep 5, 16, and 18 had infections of the suture lines used for renal biopsies, evidenced by accumulation of pus and by necrosis of the muscular wall of the abdomen. No sheep had gross lesions of renal disease nor any other significant lesions in the remainder of the carcasses or viscera.

The severity, nature, and extent of renal lesions varied considerably between sheep. Five of 7 sheep had renal lesions and, in each, the lesions appeared as groups or streaks of affected tubules. The mildest lesions consisted of fine vacuolization and hyaline droplet formation in small groups of proximal tubular epithelial cells (Fig 3). More severely affected groups of proximal tubules were lined by epithelial



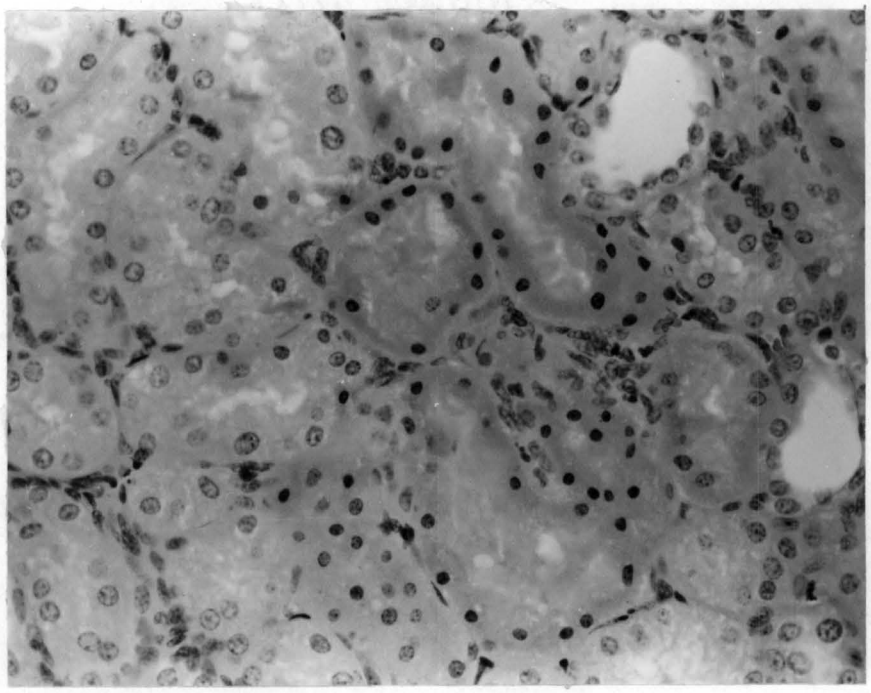
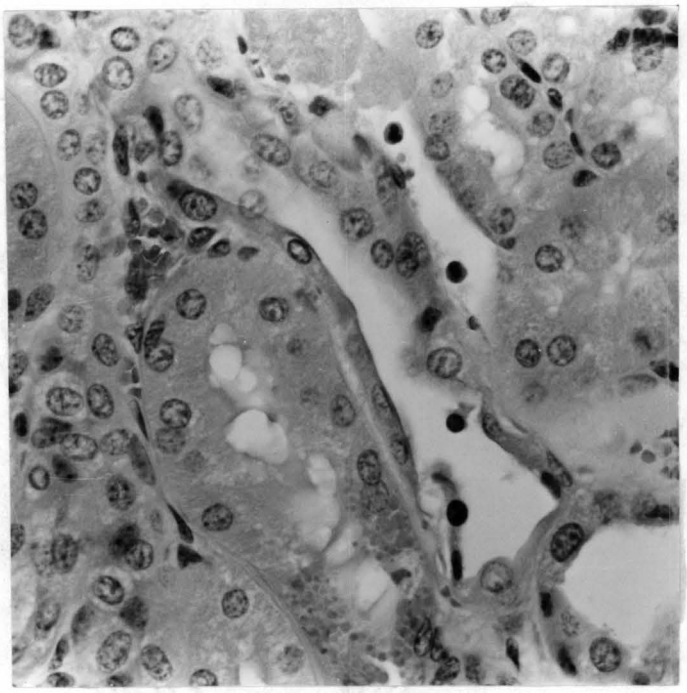


Fig 3--Proximal tubular epithelial cells from an oak leaf-poisoned sheep containing hyaline droplets. H&E stain; X40.

Fig 4--Group of proximal tubular epithelial cells which have pyknotic nuclei and deeply eosinophilic cytoplasm. H&E stain; X25.

cells which had pyknotic nuclei and deeply eosinophilic cytoplasm (Fig 4). These cells occasionally detached from the basement membrane and were free in the lumen (Fig 5). The most severely affected kidneys had severe, widespread tubular and glomerular dilatation, attenuation of tubular epithelia, and accumulation of cellular debris in tubular lumens (Fig 6). Luminal debris varied from dead cells and amorphous debris (Fig 7) to casts (Fig 8). Individual animals had varying combinations of the above lesions, but usually 1 type predominated. Whereas the lesions followed a rather typical morphological progression from tubular epithelial degeneration to necrosis, the progression was not clearly seen within a particular animal or at a particular biopsy interval. Light microscopic lesions occurred within 2 to 5 days of oak-leaf treatment (Table 6). No other significant light microscopic lesions were seen in any other tissue examined.

Ultrastructural lesions reflected those of light microscopy. Lesions progressed from cellular swelling and organelle alterations to cell death and detachment from the basement membrane. Early proximal tubular epithelial changes consisted of ribosome disaggregation from rough endoplasmic reticulum (RER), polyribosome disaggregation, cytoplasmic rarefaction, and loss of microvilli (Fig 9). Low amplitude swelling of mitochondria and mild dilatation of endoplasmic reticulum were seen also (Fig 10). Affected cells then underwent cytoplasmic contracture and either died in the tubule wall (Fig 11) or detached as intact cells (Fig 12). Such cells had dense nuclear chromatin, moderate mitochondrial swelling, severe endoplasmic reticulum swelling, and developed myelin figures. Detached cells eventually ruptured releasing cellular contents into the lumen to produce the luminal debris seen by



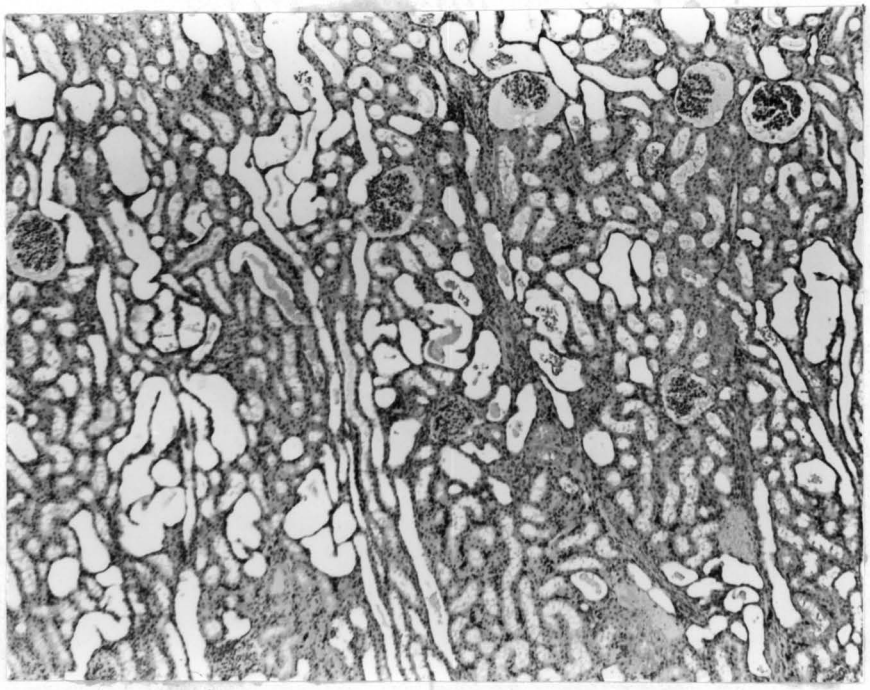
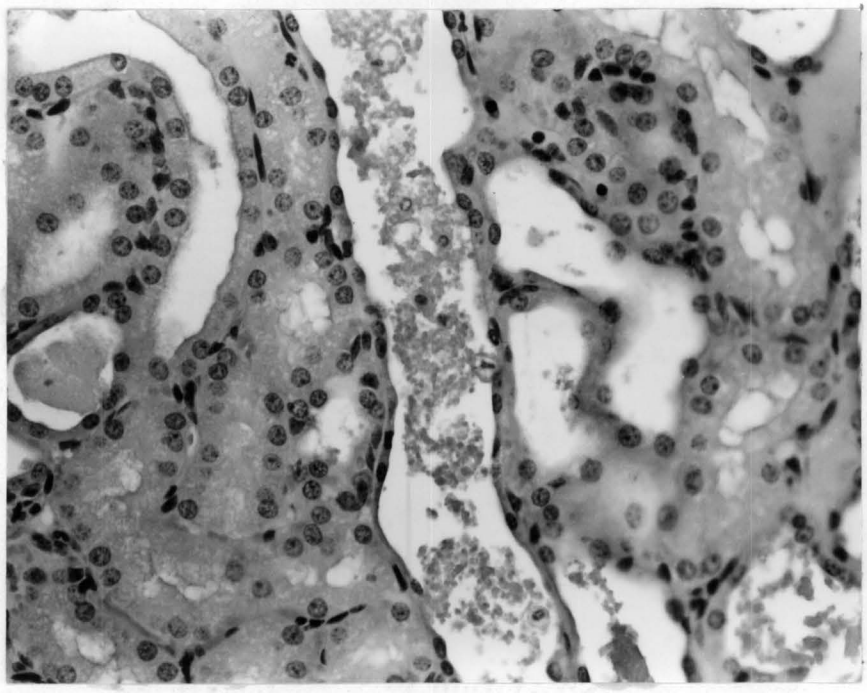


Fig 5--Dilated proximal tubule composed of flattened and detached epithelial cells. Note hyaline droplets in epithelia of adjacent tubule. H&E stain; X40.

Fig 6--Severely affected kidney demonstrating tubular and glomerular dilatation, epithelial attenuation, and luminal debris. H&E stain; X6.3.



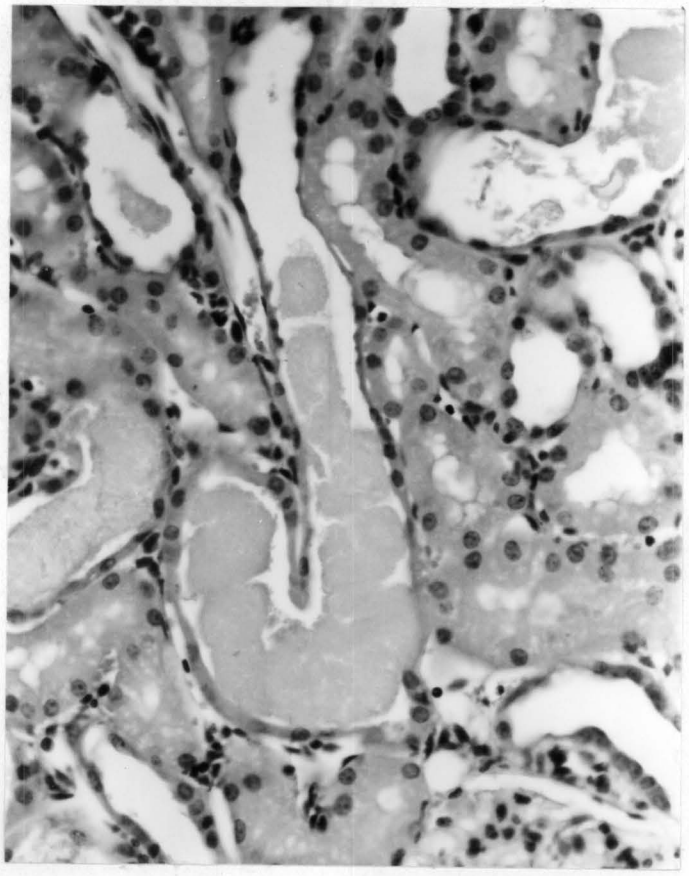


Fig 7--Tubule containing granular, amorphous debris and dead cells. H&E stain; X25.

Fig 8--Tubules containing casts. H&E stain; X25.

TABLE 6--Occurrence of light and electron microscopic renal lesions in oak leaf-treated sheep subjected to renal biopsies

Sheep	First Day of Light Microscopic Renal Lesion	First Day of Electron Microscopic Renal Lesion
5	5	5
14	5	8
15	2	5
16	5	2
17	2	2
18	NL*	NE+
19	NL	NE

*No lesion.
+Not examined.

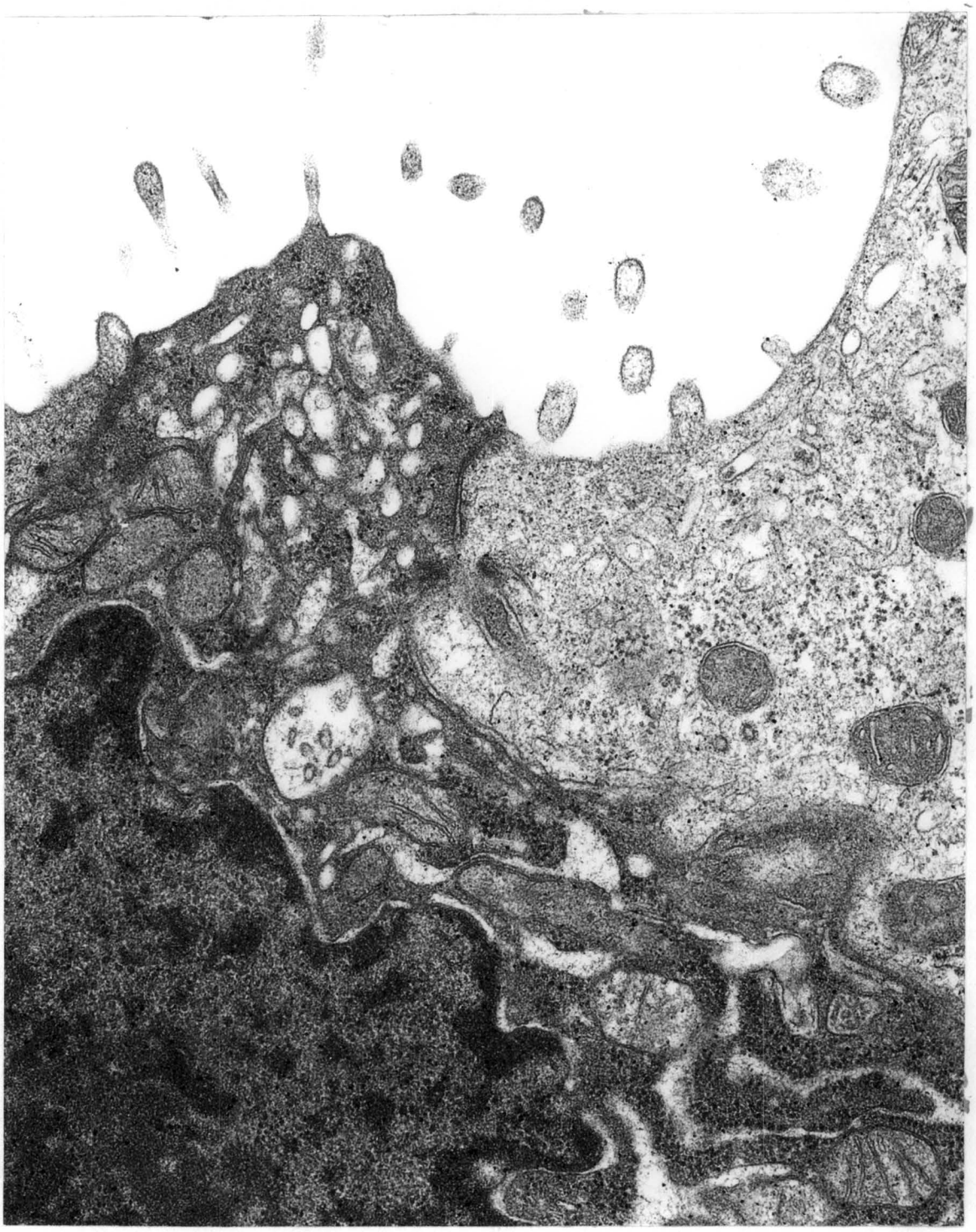


Fig 9--Proximal convoluted tubular epithelial cells with cytoplasmic rarefaction, ribosomal detachment from RER, polyribosomal disaggregation, and loss of microvilli. Uranyl acetate and lead citrate (UA/LC) stain; X42,500.

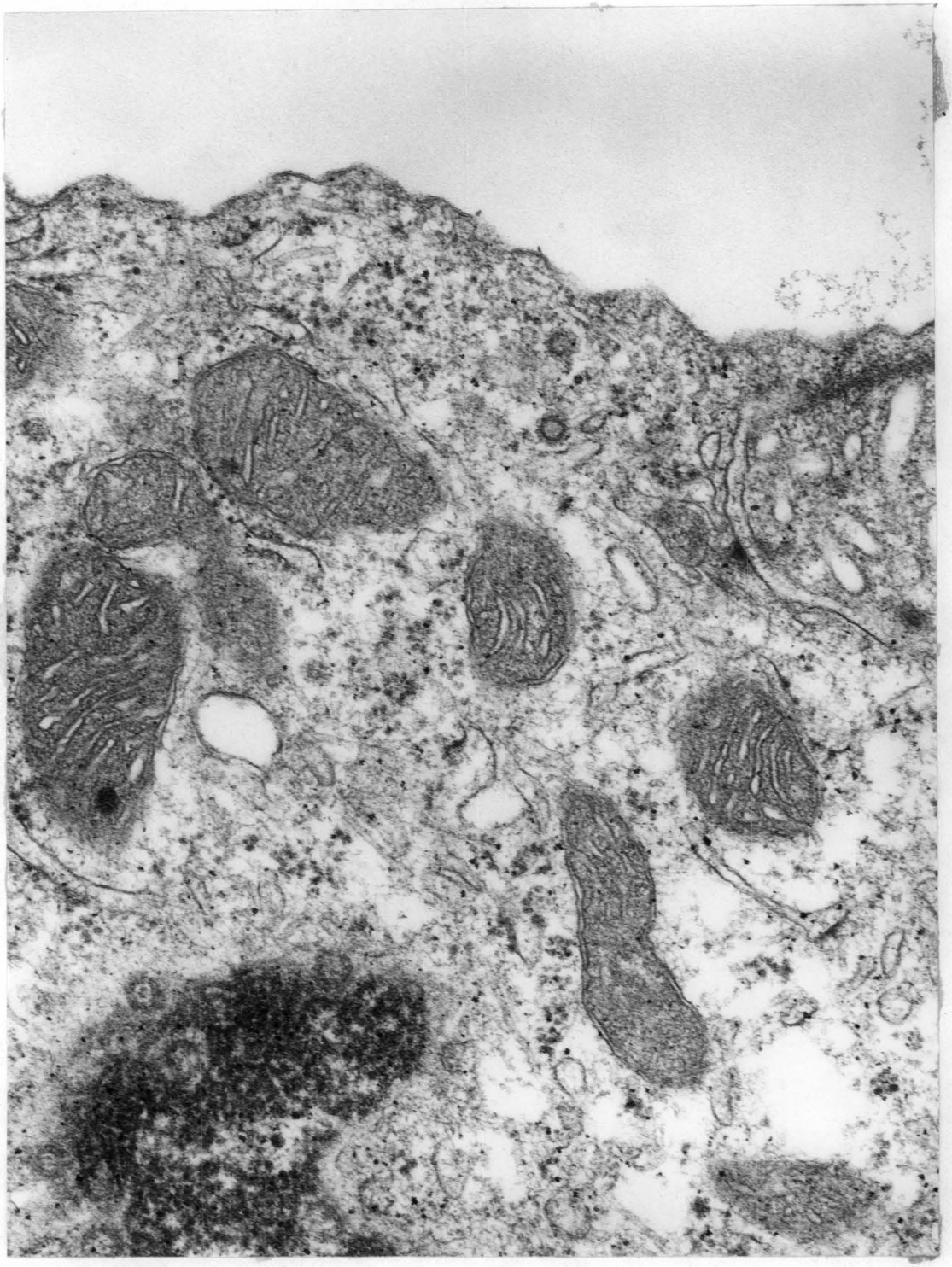


Fig 10--Proximal straight tubular epithelial cell with low-amplitude mitochondrial swelling, cytoplasmic rarefaction, and ribosomal detachment from RER. UA/LC stain; X57,500.

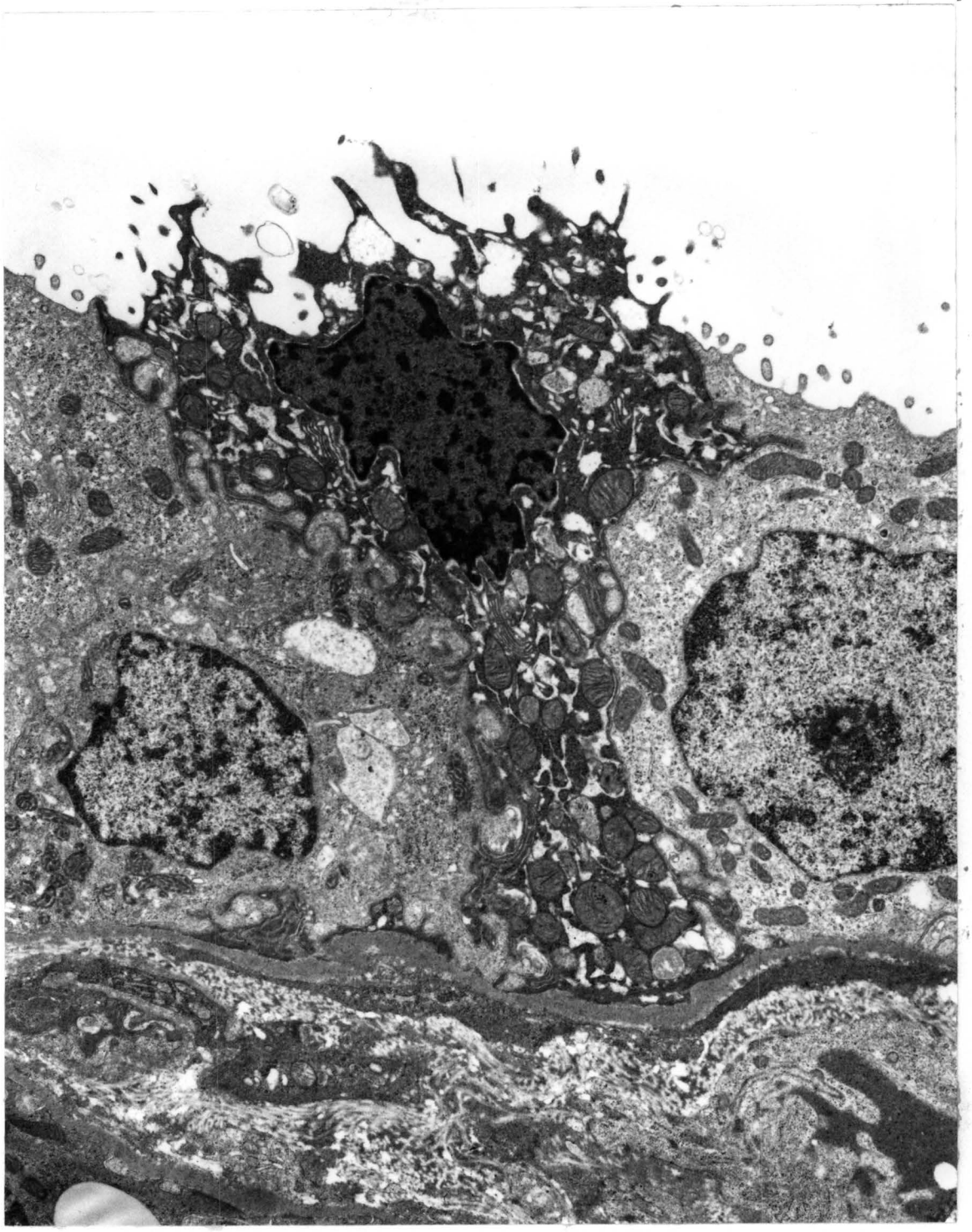


Fig 11--Proximal straight tubular epithelial cell with cytoplasmic contracture and nuclear pyknosis. UA/LC stain; X11,250.

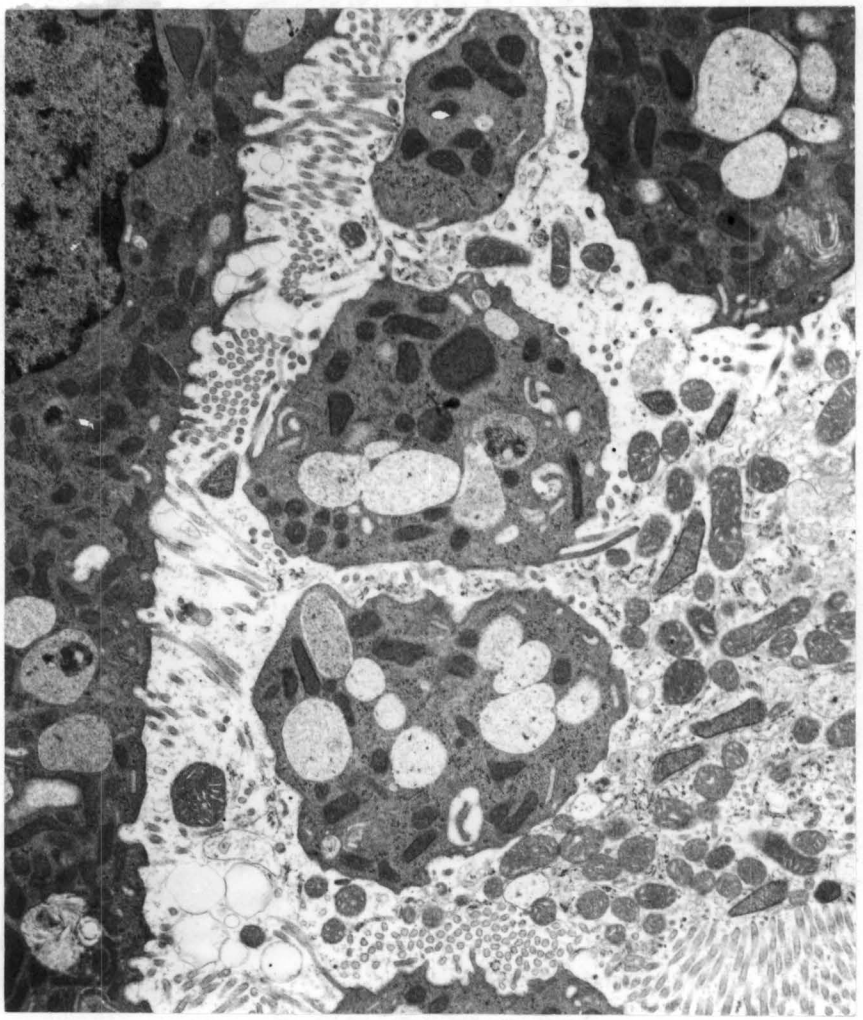


Fig 12--Detached proximal convoluted tubular epithelial cells and cellular debris in lumen of proximal convoluted tubule. UA/LC stain; X6,500.

light microscopy (Fig 13). Luminal material further degenerated to amorphous, finely granular material (Fig 14). Lesions were not seen in glomeruli or other types of tubules.

Discussion

Five sheep (Nos. 5, 14, 15, 16, 17) had decreases in serum sodium. Serum sodium values may decrease during renal disease, and this may offer an explanation for the decreases seen in sheep of the present study (69). In addition, sheep 5 had increases of hematocrit and serum protein, indicating dehydration, and increases of BUN and creatinine also, indicating renal disease or dehydration. Six sheep (Nos. 5, 14, 15, 16, 17, 18) had decreased serum potassium, and 2 of these (Nos. 5, 16) also had decreases in serum chloride. Decreases in serum potassium and chloride are seen in metabolic alkalosis and may be indicative of that condition in the sheep of the present study (69). Blood gas determinations were not performed on these sheep, however, so the validity of this interpretation cannot be determined. Four sheep (Nos. 5, 16, 18, 19) had an increased WBC suggesting that each had an active infection. This was supported in sheep 5, 16, and 18 by necropsy findings. No site of infection was seen in sheep 19, but mild inflammation associated with repeated renal biopsies may have accounted for the modest rise in WBC. Total bilirubin and SGOT rises in sheep 5 suggested hepatic or muscle disease, but this could not be confirmed by light microscopy. Hepatic lesions are not commonly described in cases of oak poisoning. The possible liver disease in this sheep may have been a result of oak-leaf toxins, anesthesia, or some unidentified factor. Similar factors may have caused the rise in SGOT seen in sheep No. 15. The high levels of

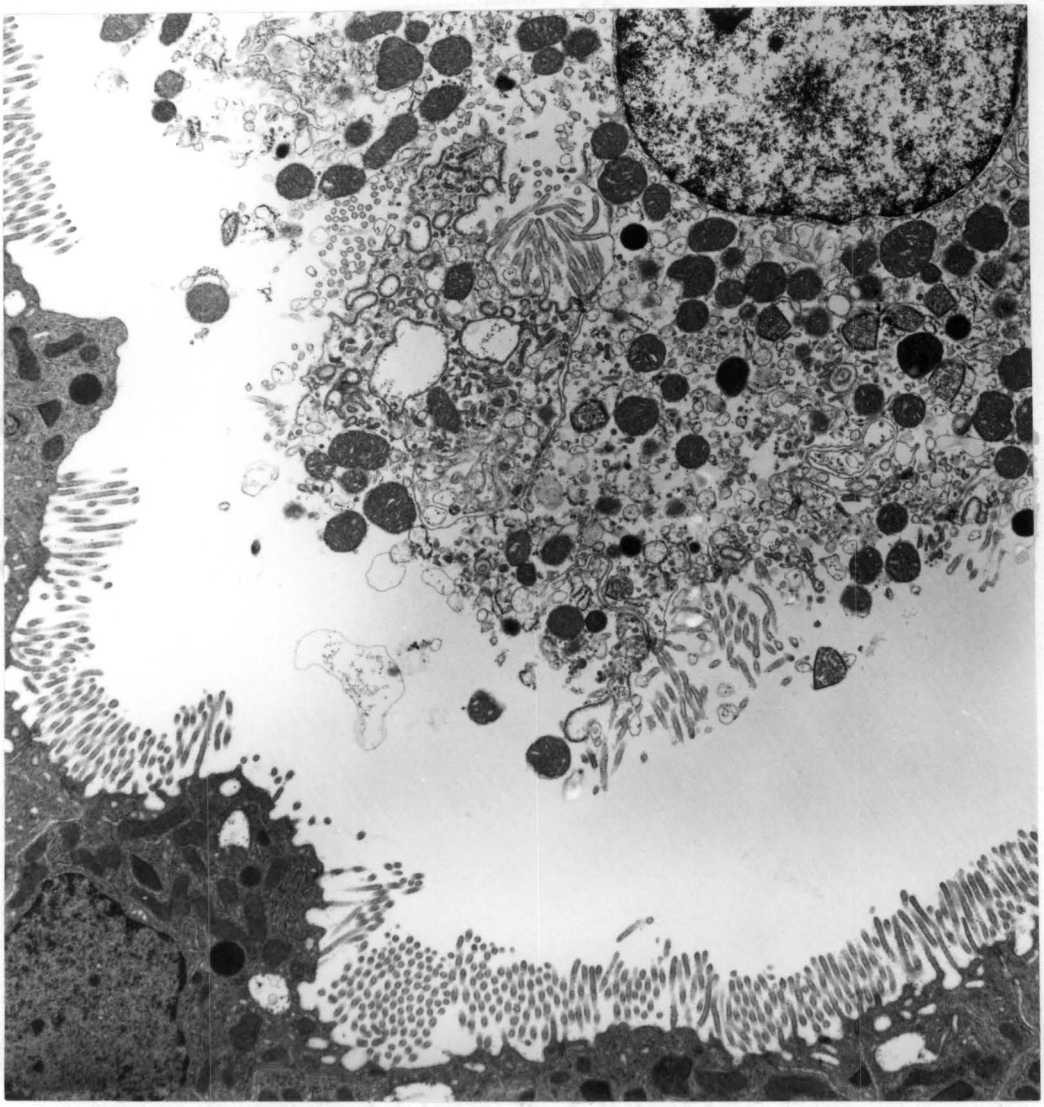


Fig 13--Ruptured proximal convoluted tubular cell in lumen of proximal convoluted tubule. Note the relative lack of mitochondrial change. UA/LC stain; X11,250.

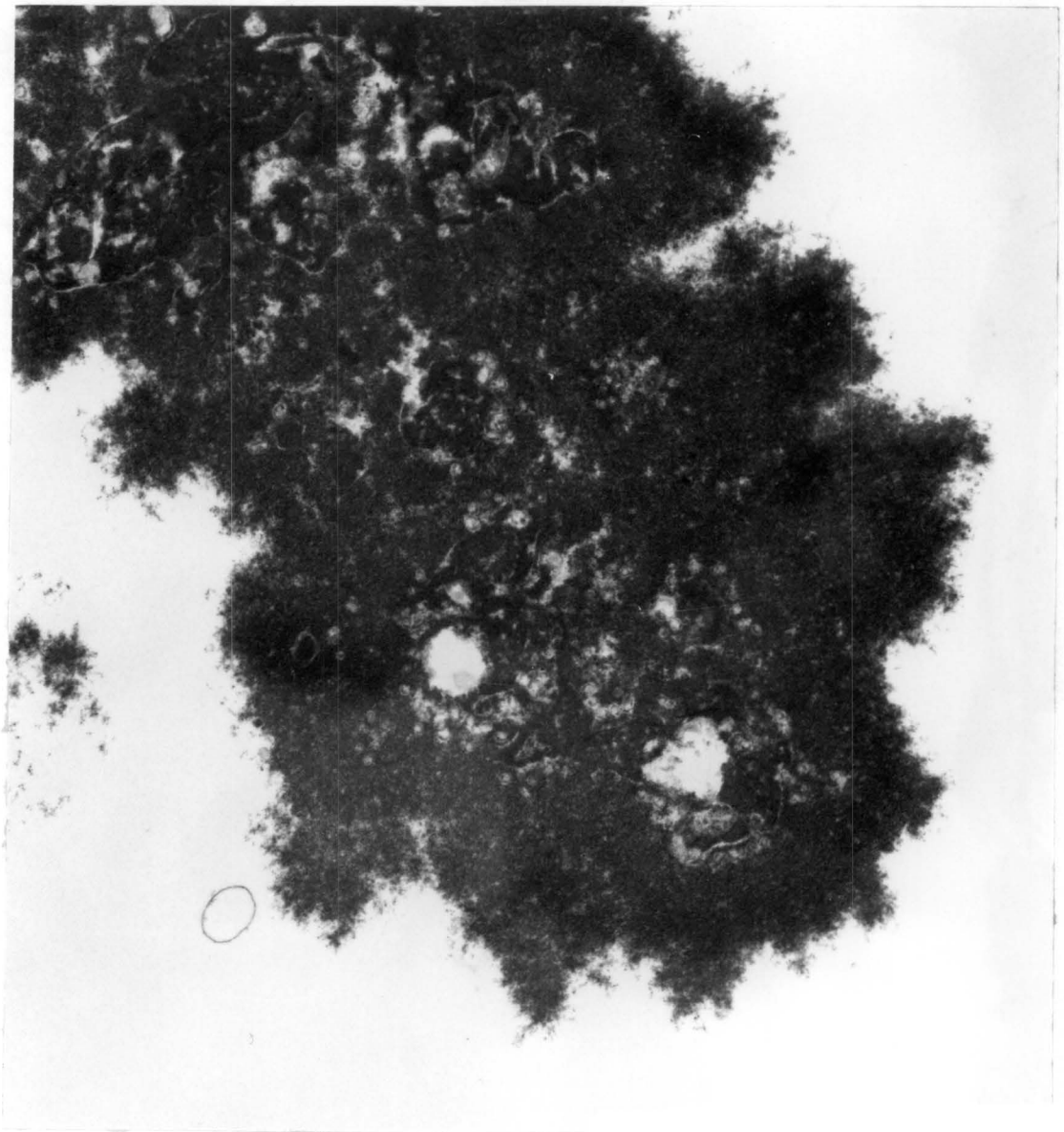


Fig 14--Granular, amorphous luminal debris. UA/LC stain; X18,750.

blood glucose seen in all sheep are possibly the result of a response to stress (69).

Light microscopic lesions in sheep of this study resembled those of oak leaf-poisoned cattle and sheep (6, 9, 17, 22, 24). Variation was seen in sheep of this experiment in regards to occurrence and severity of renal lesions. As with many toxins, not all animals will respond in exactly the same manner or to the same degree. Variations in bioactivation, absorption, detoxification, and excretion may determine whether or not the oak-leaf toxin will affect a given animal. The day on which renal lesions were first noticed varied considerably between animals and between light and electron microscopic samples of the same animal (Table 6). This disparity may reflect the small biopsy sample volume and the distribution of the renal lesion. It is conceivable that the small sample collected with the biopsy needle was not representative of the tissue in the kidney at that time. This problem is compounded by examination of an even smaller amount of tissue for electron microscopy purposes. In addition, because the renal lesion affects groups of tubules, nonrepresentative sampling is made even more likely. Similarly, the absence of a clear sequence of morphologic lesion progression in both light and electron microscopy samples of the same animal may have been affected by the same factors.

A review of the pathophysiology and pathomorphology of acute ischemic cell injury is a useful prelude to discussion of the renal lesion seen in the present study (70). Following an initial ischemic event, a rapid decrease in cellular ATP levels occurs. Simultaneously, the cell begins to utilize glycogen via anaerobic metabolism which leads to a decrease in cytoplasmic glycogen granules. This is followed by low-

amplitude swelling of mitochondria. Affected mitochondria have a dilated intermembranous space, contracted matrix, and loss of matrix granules. Microvilli are lost at this point. The next major event is disaggregation of polysomes and detachment of ribosomes from RER. The cell then begins to swell, and high-amplitude swelling of mitochondria is seen. The mitochondrial matrix swells, and amorphous densities appear. Mitochondria continue to swell to the point where the inner membrane is fragmented, and breaks occur in the outer membrane. Myelin figure formation and organelle fragmentation begin. Finally, lysosomes swell and rupture.

Two features of the ultrastructural lesions of sheep in the present study differ from those of ischemic cell injury. First, glycogen granules are prominent in the cytoplasm of affected cells. This suggests that the cell has not switched to anaerobic metabolism. Secondly, mitochondria in affected cells of oak-poisoned sheep do not undergo severe structural changes. Low-amplitude swelling is the only alteration seen in mitochondria of affected cells. These 2 features, when considered together, suggest that cellular energy production organelles and pathways are not the primary target of the oak-leaf toxin. This would also suggest that ischemia is not a major mechanism in the pathogenesis of the oak-induced lesion because a loss of energy production capability plays a central role in ischemia. The ultrastructural lesions in these sheep do not provide a basis for much speculation as to where or how the oak toxin acts in the cell. However, the apparent loss of plasma membrane functional integrity suggests that a component of the membrane may be the target of the toxin. Alternatively, alterations in protein-synthesizing organelles seen in these sheep suggest that they

could also be a target of the toxin. In support of the latter possibility, dissociation of ribosomes from RER has been described in hepatocytes of rabbits given tannic acid, the presumed toxin in oak leaves (34). Decreased protein production might lead to a decrease in membrane functional integrity.

CHAPTER VIII

Conclusions

This study has addressed each of the stated purposes of this investigation, and certain conclusions can be made. The conclusions are:

1. Rabbits can be poisoned by oak-leaf administration, develop renal lesions, and may have limited use as an experimental model of oak-leaf toxicity in larger herbivores.

2. Mice could not be poisoned by free-choice consumption of an oak-leaf ration.

3. Sheep are susceptible to poisoning by oak leaves and develop renal lesions.

4. One of 5 sheep had decreases in glomerular filtration rate and total renal blood flow as a result of poisoning by oak leaves.

5. A defined sequence of light and electron microscopic lesions occurs in proximal tubular epithelial cells as a result of poisoning by oak leaves.

6. The renal lesion resulting from oak-leaf toxicity in sheep appears to be of a direct tubulotoxic nature.

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APPENDIX A

TABLE 7--Hematologic values of oak leaf-treated sheep subjected to clearance studies

		T.P. (g/dl)	WBC (X1,000)	Hgb. (g/dl)	Hct. (%)	Seg (%/abs*)	Band (%/abs)	Lym (%/abs)	Mono (%/abs)	Eosin (%/abs)	Baso (%/abs)
Sheep 6											
Presurgical		5.6	6.6	11.9	33.0	34/2,244	0	62/4,092	4/264	0	0
Control	1	7.3	9.1	12.9	39.0	23/2,093	0	73/6,643	0	4/364	0
Control	2	8.3	8.7	14.1	41.5	65/5,655	0	32/2,784	0	2/174	0
Day	2	9.0	13.1	13.5	43.5	72/9,432	0	25/3,275	3/393	0	0
	5	7.7	15.9	11.5	35.0	79/12,561	0	19/3,021	0	3/318	0
	8	7.6	15.9	12.1	37.5	79/12,561	0	17/2,703	3/477	1/159	0
Sheep 7											
Presurgical		6.2	8.3	11.3	34.0	42/3,486	0	56/4,648	2/166	0	0
Control	1	7.6	7.3	12.9	38.0	44/3,212	0	49/3,577	3/219	3/219	1/73
Control	2	7.8	14.8	12.9	42.0	67/9,916	0	33/4,884	0	0	0
Day	2	7.4	8.6	12.1	38.0	40/3,440	0	58/4,988	2/172	0	0
	5	7.3	8.8	10.6	35.0	47/4,136	0	50/4,400	1/88	2/176	0
	8	7.5	9.2	9.2	30.5	51/4,692	0	43/3,956	5/460	1/92	0
	11	7.3	8.3	8.2	26.5	51/4,233	0	46/3,818	2/166	1/83	0
	14	7.0	8.6	7.9	23.5	46/3,956	0	51/4,386	4/344	0	0
	17	7.1	7.5	6.9	21.0	30/2,250	0	66/4,950	2/150	2/150	0
	20	6.6	6.2	6.1	19.5	30/1,860	0	62/3,844	6/372	2/124	0
Biopsy		7.1	8.2	5.8	17.5	56/4,592	0	40/3,280	4/328	0	0

TABLE 7 (continued)

		T.P. (g/dl)	WBC (X1,000)	Hgb. (g/dl)	Hct. (%)	Seg (%/abs)	Band (%/abs)	Lym (%/abs)	Mono (%/abs)	Eosin (%/abs)	Baso (%/abs)
Sheep 8											
Presurgical		6.2	7.0	13.5	31.0	24/1,680	0	67/4,690	9/630	0	0
Control	1	6.9	7.1	12.8	37.5	23/1,633	0	69/4,899	5/355	1/71	0
Control	2	--	11.9	11.4	34.0	48/5,712	0	49/5,831	3/351	0	0
Day	2	7.0	8.6	11.6	35.0	24/2,064	0	71/6,106	5/430	0	0
	5	6.3	19.7	10.0	29.4	41/8,077	0	55/10,835	3/591	0	1/197
	8	6.8	20.8	8.5	28.0	53/11,024	0	47/9,776	1/208	0	0
	11	6.5	18.9	8.2	24.0	30/5,670	0	70/13,230	0	0	0
Biopsy		7.0	18.6	8.6	26.5	47/8,742	5/930	47/8,742	1/186	0	0
Sheep 11											
Presurgical		6.4	6.1	10.1	26.5	20/1,220	0	75/4,575	4/244	1/61	0
Control	1	--	9.4	10.4	30.5	74/--	0	26/--	0	0	0
Control	2	7.4	10.0	10.1	34.0	61/6,100	1/100	36/3,600	1/100	1/100	0
Day	2	8.1	11.6	9.6	32.5	65/7,540	0	35/4,060	0	0	0
	5	6.9	12.5	8.8	26.0	63/7,875	0	37/4,625	0	0	0
	8	7.0	12.2	--	--	71/8,662	0	25/3,050	4/488	0	0
Biopsy		7.1	7.3	7.5	22.0	66/4,818	0	33/2,409	0	0	1/73

TABLE 7 (continued)

		T.P. (g/dl)	WBC (X1,000)	Hgb. (g/dl)	Hct. (%)	Seg (%/abs)	Band (%/abs)	Lym (%/abs)	Mono (%/abs)	Eosin (%/abs)	Baso (%/abs)
Sheep 13											
Presurgical		___	5.1	12.0	35.5	29/1,479	0	71/3,621	0	0	0
Control	1	--	10.2	11.3	33.5	58/5,916	0	39/3,978	2/204	1/102	0
Control	2	6.8	16.0	9.6	32.0	72/11,520	0	19/3,040	7/1,120	1/160	1/160
Day	2	7.3	8.4	11.2	32.0	48/4,032	0	49/4,116	1/84	2/168	0
	5	7.0	8.3	8.4	29.0	50/4,150	0	49/4,067	0	1/83	0
	8	7.1	10.0	9.5	29.0	61/6,100	0	37/3,700	2/200	0	0
Biopsy		6.9	10.7	9.9	28.0	60/6,420	0	39/4,173	1/107	0	0

*Absolute.

TABLE 8--Serum chemistry values of oak leaf-treated sheep subjected to clearance studies

		Alb. (g/dl)	Bili. T. (mg/dl)	BUN (mg/dl)	Ca (mEq/L)	Cl (mEq/L)	Creat. (mg/dl)	Glucose (mg/dl)	Na (mEq/L)	K (mEq/L)	Osm. (mmol/kg)	Phos. (mEq/L)	SGOT (IU/L)
Sheep 6													
Presurgical		3.0	0.3	22	9.0	102	0.8	68	149	4.6	291	6.3	37
Control	1	2.6	0.1	19	9.8	103	0.8	60	146	4.3	296	5.2	26
Control	2	2.7	0.1	27	10.6	106	0.8	30	148	3.6	302	5.2	24
Day	2	2.3	0.2	70	10.8	88	2.6	55	112	3.2	271	6.7	19
	5	3.2	0.3	35	9.7	106	1.2	45	132	3.0	278	8.7	60
	8	2.6	1.8	29	8.6	95	2.2	39	128	2.7	272	7.4	819
Sheep 7													
Presurgical		3.3	0.2	19	10.0	91	0.8	115	127	4.3	305	6.3	32
Control	1	3.0	0.2	19	10.0	104	0.8	115	144	4.4	296	6.3	32
Control	2	3.1	0.5	17	9.3	99	1.0	46	144	3.3	293	1.8	36
Day	2	3.1	0.3	4	10.2	103	0.5	61	143	4.5	289	4.2	35
	5	3.6	0.2	5	12.1	101	0.8	59	144	4.1	281	4.1	22
	8	2.5	0.3	7	9.2	104	0.6	54	143	4.0	292	3.3	20
	11	1.7	0.4	4	7.6	98	0.6	40	137	4.2	242	2.9	15
	14	2.3	0.2	6	9.1	99	0.6	59	144	4.4	279	3.3	18
	17	2.3	0.2	6	6.6	101	0.8	68	143	4.1	292	3.7	16
	20	1.9	0.1	4	6.7	98	0.6	58	141	3.9	268	3.8	22
Biopsy		1.8	0.3	11	7.5	100	0.7	58	146	4.7	295	4.6	18

TABLE 8 (continued)

		Alb. (g/dl)	Bili. T. (mg/dl)	BUN (mg/dl)	Ca (mEq/L)	Cl (mEq/L)	Creat. (mg/dl)	Glucose (mg/dl)	Na (mEq/L)	K (mEq/L)	Osm. (mmol/kg)	Phos. (mEq/L)	SGOT (IU/L)
Sheep 8													
Presurgical		3.1	0.2	26	9.3	97	0.7	88	139	4.4	286	5.6	41
Control	1	2.9	0.2	26	9.3	96	0.7	88	140	4.4	280	5.6	41
Control	2	2.9	0.1	20	9.3	100	0.5	40	151	4.8	291	5.7	23
Day	2	2.8	0.1	14	10.6	103	0.4	56	147	4.3	291	5.0	24
	5	4.4	0.2	22	10.9	128	0.8	63	175	5.4	363	7.6	78
	8	2.6	0.1	15	9.2	103	0.3	56	150	5.0	292	6.1	28
	11	2.4	0.3	16	8.8	100	0.4	56	150	4.9	291	6.1	24
Biopsy		3.7	0.3	9	8.3	103	0.5	90	142	4.7	279	4.0	23
Sheep 11													
Presurgical		2.3	0.1	1	8.7	101	1.0	103	152	4.9	301	7.4	33
Control	1	2.9	0.6	16	8.4	103	0.7	42	153	4.4	287	5.7	30
Control	2	2.4	0.5	12	8.6	96	0.7	45	157	4.5	288	6.1	25
Day	2	2.3	0.5	3	8.6	95	0.6	48	142	3.7	273	4.9	26
	5	2.4	0.4	1	8.5	103	0.7	42	144	3.8	271	4.3	23
	8	2.4	0.2	1	8.8	101	0.9	47	142	3.4	268	4.2	17
Biopsy		2.1	0.1	5	8.2	98	1.1	59	136	3.7	258	4.7	19

TABLE 8 (continued)

		Alb. (g/dl)	Bili. T. (mg/dl)	BUN (mg/dl)	Ca (mEq/L)	Cl (mEq/L)	Creat. (mg/dl)	Glucose (mg/dl)	Na (mEq/L)	K (mEq/L)	Osm. (mmol/kg)	Phos. (mEq/L)	SGOT (IU/L)
Sheep 13													
Presurgical		3.0	0.7	9	9.8	97	0.9	79	149	5.8	286	5.2	30
Control	1	3.1	0.2	13	9.5	105	0.9	34	159	4.6	296	5.5	35
Control	2	2.7	0.5	13	8.8	104	0.8	11	151	4.0	278	7.4	33
Day	2	2.9	0.4	3	8.6	105	0.6	46	142	3.8	273	5.8	19
	5	2.5	0.2	2	9.0	105	0.6	40	148	3.5	275	5.7	21
	8	3.0	0.2	3	8.7	106	0.7	39	144	3.8	276	4.8	18
Biopsy		3.1	0.3	2	8.2	109	0.7	60	148	4.5	277	4.6	18

TABLE 9--Urine chemistry values of oak leaf-treated sheep subjected to clearance studies

		Specific Gravity	Creatinine (mg/dl)	Na (mEq/L)	Osmolality (mmol/kg)
Sheep 6					
Control	1	1.050	164	1	1,362
Control	2	1.029	120	1	415
Day	2	1.015	84	1	193
	5	1.011	34	< 1	69
	8	1.011	56	3	1,047
Sheep 7					
Control	1	1.054	532	6	1,588
Control	2	1.039	312	2	972
Day	2	1.025	96	< 1	441
	5	1.027	44	< 1	670
	8	1.052	384	< 1	601
	11	1.031	224	< 1	680
	14	1.016	112	< 1	381
	17	1.021	146	< 1	365
	20	1.007	56	< 1	163
Sheep 8					
Control	1	1.008	38	< 1	237
Control	2	1.039	72	80	1,383
Day	2	1.037	114	< 1	988
	5	1.005	4	5	123
	8	1.036	60	10	1,130
	11	1.030	44	10	918
Sheep 11					
Control	1	1.064	415	7	2,050
Control	2	1.012	66	26	325
Day	2	1.015	69	1	216
	5	1.005	26	2	77
	8	1.006	48	< 1	133
Biopsy		1.029	152	< 1	487

TABLE 9 (continued)

		Specific Gravity	Creatinine (mg/dl)	Na (mEq/L)	Osmolality (mmol/kg)
Sheep 13					
Control	1	--	--	--	--
Control	2	1.040	193	110	1,297
Day	2	1.013	51	< 1	233
	5	--	--	--	--
	8	1.007	34	< 1	106
Biopsy		1.020	140	< 1	524

TABLE 10--Clinical pathology calculations for oak leaf-treated sheep subjected to clearance studies

		Fe_{Na}	U_{Cr}/P_{Cr}	U_{Osm}/P_{Osm}
Sheep 6				
Control	1	0.003	205	4.6
Control	2	0.005	150	1.4
Day	2	0.028	32	0.7
	5	0	28	0.2
	8	0.100	26	3.8
Sheep 7				
Control	1	0.006	665	5.4
Control	2	0.005	312	3.3
Day	2	0	192	1.5
	5	0	55	2.4
	8	0	640	2.1
	11	0	373	2.8
	14	0	187	1.4
	17	0	183	1.3
	20	0	93	0.6
Sheep 8				
Control	1	0	54	0.8
Control	2	0.400	144	4.8
Day	2	0	285	3.4
	5	0.600	5	0.3
	8	0.033	200	3.9
	11	0.100	110	3.2
Sheep 11				
Control	1	0.008	593	7.1
Control	2	0.200	94	1.1
Day	2	0.006	115	0.8
	5	0	37	0.3
	8	0	53	0.5
Sheep 13				
Control	1	--	--	--
Control	2	0.300	241	4.7
Day	2	0	85	0.9
	5	--	--	--
	8	0	49	0.4

APPENDIX B

TABLE 11--Hematologic values of oak leaf-treated sheep subjected to renal biopsies

		T.P. (g/dl)	WBC (X1,000)	Hgb. (g/dl)	Hct. (%)	Seg (%/abs*)	Band (%/abs)	Lym (%/abs)	Mono (%/abs)	Eosin (%/abs)
Sheep 5										
Presurgical		6.1	4.6	11.5	34.0	39/1,794	0	57/2,622	1/46	3/138
Day	2	8.3	7.5	16.5	51.5	58/4,350	0	39/2,925	3/225	0
	5	9.1	13.4	17.5	56.0	88/11,792	1/134	9/1,206	2/268	0
	8	9.2	16.0	18.3	58.0	78/12,488	7/1,120	12/1,920	3/480	0
Sheep 14										
Presurgical		6.2	3.8	9.8	28.0	25/950	0	75/2,850	0	0
Day	2	8.2	4.8	10.0	30.5	37/1,776	0	55/2,640	8/384	0
	5	8.4	5.0	11.9	35.0	48/2,400	0	48/2,400	4/200	0
	8	9.0	7.7	12.0	35.0	78/6,006	0	20/1,540	2/154	0
	11	Sample destroyed								
Sheep 15										
Presurgical		6.4	4.9	8.5	25.0	18/882	0	82/4,018	0	0
Day	2	8.7	7.9	10.8	32.5	57/4,503	0	39/3,081	3/237	1/70
	5	9.0	5.3	10.2	30.5	38/2,014	0	52/2,756	10/530	0
	8	8.8	10.4	10.7	32.0	75/7,800	0	24/2,496	1/104	0
	11	Sample destroyed								

TABLE 11 (continued)

		T.P. (g/dl)	WBC (X1,000)	Hgb. (g/dl)	Hct. (%)	Seg (%/abs)	Band (%/abs)	Lym (%/abs)	Mono (%/abs)	Eosin (%/abs)
Sheep 16										
Presurgical		6.2	5.3	9.7	29.0	35/1,855	0	65/3,445	0	0
Day	2	8.2	5.9	10.6	24.0	43/2,537	0	55/3,245	2/118	0
	5	7.5	6.1	9.9	30.0	61/3,721	0	34/2,074	5/305	0
	8	8.5	9.8	10.9	33.0	71/6,958	0	28/2,744	1/98	0
	11	9.2	31.2	14.9	44.0	94/29,328	0	4/1,248	2/624	0
Sheep 17										
Presurgical		6.6	4.5	10.3	29.5	23/1,035	0	75/3,375	2/90	0
Day	2	8.0	9.0	10.5	32.0	59/5,310	0	40/3,600	1/90	0
	5	8.5	9.1	9.6	30.5	52/4,732	0	43/3,913	5/455	0
	8	9.0	10.6	10.2	31.0	73/7,738	0	23/2,438	3/318	1/106
	11	Sample destroyed								
Sheep 18										
Presurgical		7.0	7.6	10.9	33.0	25/1,900	0	74/5,624	1/76	0
Day	2	7.0	11.3	9.9	29.0	22/2,486	0	75/8,475	3/339	0
	5	7.2	5.9	9.3	29.0	22/1,298	0	71/4,189	7/413	0
	8	8.0	23.8	10.7	35.0	96/18,088	0	22/5,236	2/476	0

TABLE 11 (continued)

	T.P. (g/dl)	WBC (X1,000)	Hgb. (g/dl)	Hct. (%)	Seg (%/abs)	Band (%/abs)	Lym (%/abs)	Mono (%/abs)	Eosin (%/abs)
Sheep 19									
Presurgical	6.8	7.6	10.3	31.0	24/1,824	0	70/5,320	3/228	3/228
Day 2	7.0	9.9	10.5	27.0	28/2,772	0	65/6,435	5/495	2/198
5	7.6	8.2	9.0	26.0	26/2,132	0	67/5,494	5/410	2/164
8	6.7	12.1	7.8	22.0	52/6,292	0	45/5,445	2/242	1/121

*Absolute.

TABLE 12--Serum chemistry values of oak leaf-treated sheep subjected to renal biopsies

		Alb. (g/dl)	Bili. T. (mg/dl)	BUN (mg/dl)	Ca (mEq/L)	Cl (mEq/L)	Creat. (mg/dl)	Glucose (mg/dl)	Na (mEq/L)	K (mEq/L)	Osm. (mmol/kg)	Phos. (mEq/L)	SGOT (IU/L)
Sheep 5													
Presurgical		3.2	0.3	29	9.6	100	0.7	126	144	4.7	295	4.9	31
Day	2	3.7	0.3	61	9.4	95	1.0	51	142	3.4	296	4.3	41
	5	3.8	0.5	133	14.7	93	4.0	97	132	2.6	324	3.8	1,165
	8	3.4	0.9	185	18.2	86	9.8	77	128	3.1	343	6.8	1,547
Sheep 14													
Presurgical		2.7	0.5	8	7.4	96	0.9	87	146	4.4	297	8.7	33
Day	2	2.5	0.4	9	7.9	99	1.1	81	144	3.8	289	6.2	37
	5	2.5	0.5	6	8.5	98	0.8	100	135	3.3	274	5.3	40
	8	3.3	0.2	11	8.3	98	1.3	126	131	2.8	260	5.9	42
	11	Sample destroyed											
Sheep 15													
Presurgical		3.2	0.3	14	7.7	96	1.0	108	149	4.4	290	10.6	36
Day	2	2.6	0.5	12	8.7	92	0.9	139	144	3.4	299	6.9	27
	5	2.6	0.1	12	8.8	102	1.3	228	140	3.5	297	4.7	55
	8	3.0	0.3	20	8.5	100	2.1	154	130	2.8	267	8.2	728
	11	Sample destroyed											

TABLE 12 (continued)

		Alb. (g/dl)	Bili. T. (mg/dl)	BUN (mg/dl)	Ca (mEq/L)	Cl (mEq/L)	Creat. (mg/dl)	Glucose (mg/dl)	Na (mEq/L)	K (mEq/L)	Osm. (mmol/kg)	Phos. (mEq/L)	SGOT (IU/L)
Sheep 16													
Presurgical		3.3	0.3	15	8.6	106	0.8	120	150	4.3	291	7.7	41
Day	2	2.9	0.2	11	9.0	98	0.9	115	147	3.5	301	5.9	27
	5	2.8	0.2	18	9.3	100	1.6	223	133	3.1	288	5.5	37
	8	3.6	0.3	30	9.7	99	2.5	240	125	3.0	274	4.5	38
	11	3.5	0.4	55	12.4	88	6.2	108	127	3.7	286	8.7	60
Sheep 17													
Presurgical		3.0	0.2	18	8.6	103	1.0	62	158	4.6	294	9.7	43
Day	2	2.5	0.1	11	8.9	100	0.9	66	145	4.1	293	4.6	25
	5	2.2	0.3	3	7.9	107	0.8	94	142	3.2	286	5.3	31
	8	2.7	0.2	3	9.6	106	0.8	83	137	3.0	275	6.7	50
	11	Sample destroyed											

TABLE 12 (continued)

	Alb. (g/dl)	Bili. T. (mg/dl)	BUN (mg/dl)	Ca (mEq/L)	Cl (mEq/L)	Creat. (mg/dl)	Glucose (mg/dl)	Na (mEq/L)	K (mEq/L)	Osm. (mmol/kg)	Phos. (mEq/L)	SGOT (IU/L)
Sheep 18												
Presurgical	3.0	0.2	16	8.6	98	0.8	123	146	4.2	283	5.9	69
Day 2	2.6	0.1	15	8.7	102	0.8	91	149	4.2	306	6.6	38
5	2.2	0.1	12	8.5	98	1.5	102	147	4.5	298	4.9	37
8	2.7	0.1	10	8.8	104	1.2	13	145	3.7	282	5.0	55
Sheep 19												
Presurgical	2.4	0.2	8	8.1	95	0.6	82	145	4.3	283	8.0	41
Day 2	1.9	0.1	8	8.4	101	0.6	107	150	4.4	305	7.5	37
5	2.1	0.1	5	7.4	101	0.6	108	153	4.0	299	7.1	52
8	2.4	0.1	12	8.6	97	0.8	149	149	4.3	293	6.8	58

VITA 2

Mark Andrew Zimmer

Candidate for the Degree of

Doctor of Philosophy

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